

**Phthalocyanine-nanoparticle conjugates for
photodynamic therapy of cancer and
phototransformation of organic pollutants**

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

DOCTOR OF PHILOSOPHY

Of

RHODES UNIVERSITY

By

PHINDILE BRENDA KHOZA

November 2014

DEDICATIONS

This work is dedicated to my mother, Buhlebezintombi Angelina Khoza, how much and why I am so grateful to you is a thesis on its own. Thank you *Ngomane*.

ACKNOWLEDGEMENTS

It is with greatest gratitude that I thank the following people, without whom none of this would have been possible:

First and foremost, my supervisor: Prof Tebello Nyokong, thank you so much Prof for your guidance, I am forever in your debt. Dr Edith Antunes, words cannot begin to describe how much I am grateful to you, you will always have a special place in this heart of mine. To people in Switzerland, thank you to Dr Norbet Lange for hosting me; Martha Coupin, thank you for everything. My appreciation goes to Ivy Ndhundhuma, Dr Aletta Karsten for hosting me at CSIR, and helping me with the cell studies. Dr Prinsloo and Shane, in Biochem, thank you for your assistance and kindness.

S22, I honestly cannot name each and every one of you, it's been real. Rhodes University, Chemistry department thank you for hosting me.

A special thanks goes out to my family, my mother, I have no idea what I would have been without you, thank you for everything, you mean a lot to me. My siblings, and my wonderful father, thank you for your support. To my son, Sbongakonke, I am grateful for you and I love you too much. My cousins, a special thanks goes to the Welas, Sthe and her husband, you people are too kind, thank you.

ABSTRACT

The synthesis and extensive spectroscopical characterization of novel phthalocyanines are reported. The new compounds were characterized by elemental analysis, FT-IR, ^1H NMR, mass spectrometry and UV-Vis spectroscopy. The new phthalocyanines showed remarkable photophysicochemical behaviour. The novel phthalocyanines were then conjugated to nanoparticles, silver and ZnO. The coupling of the novel Pcs to nanoparticles was through covalent bonding and ligand exchange. These conjugates were supported onto electrospun polystyrene fibers and chitosan microbeads for use as photocatalysts. The efficiency of the immobilized Pcs and Pc-nanoparticles was assessed by the phototransformation of organic pollutants, methyl orange and Rhodamine 6G as model dyes. Upon conjugating phthalocyanines to nanoparticles, there was a great increase in the rate of photodegradation of the model dyes.

The photodynamic activity of the novel phthalocyanines upon conjugating to nanoparticles and selected targeting agents is also reported. The targeting agents employed in this study are folic acid and polylysine. Conjugating the phthalocyanines to folic acid or polylysine improved the solubility of the phthalocyanines in aqueous media. The potency of the conjugates was investigated on breast (MCF-7), prostate and melanoma cancer cell lines. The phthalocyanines showed no toxicity in the absence of light. However, upon illumination, a concentration dependent cellular decrease was observed.

TABLE OF CONTENTS

TITLE PAGE	i
DEDICATIONS	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
TABLE OF CONTENTS	v
LIST OF SYMBOLS.....	xi
LIST OF ABBREVIATIONS.....	xii
LIST OF FIGURES.....	xiv
LIST OF SCHEMES	xxii
LIST OF TABLES.....	xxiv
CHAPTER 1	1
INTRODUCTION	1
1.1 Nanoparticles	2
1.1.1 Silver nanoparticles (AgNPs).....	2
1.1.2 Zinc Oxide (ZnO) nanoparticles	4
1.2 Background on Phthalocyanines.....	7
1.2.1 Structure, general synthesis and applications.....	7
1.2.2 Synthesis of peripherally (β) and non-peripherally (α) substituted phthalocyanines	10
1.2.3 Phthalocyanines synthesized in this work	12
1.2.4 Origins of spectra in phthalocyanines	14
1.2.5 Aggregation in phthalocyanines	16
1.2.6 Photophysics and photochemistry of phthalocyanines.....	17
1.2.6.1 Fluorescence quantum yields and lifetimes	18

1.2.6.2	Triplet quantum yields and lifetimes	19
1.2.6.3	Singlet oxygen quantum yields	20
1.2.7	Photophysical properties of phthalocyanines in the presence nanoparticles.	22
1.3	Phthalocyanines in photodynamic therapy of cancer	25
1.3.1.	Folic acid	28
1.3.2	Polymer conjugates	30
1.3.3	Cancer cells employed in this work	32
1.4	Phthalocyanines as photocatalysts	33
1.4.1	Electrospun fibers preparation	34
1.4.2	Chitosan microbeads	36
1.4.3	Brief description of dye pollutants employed in this work	39
1.5	Summary of Aims	41
CHAPTER 2		43
EXPERIMENTAL SECTION		43
2.1	Materials	44
2.1.1	Solvents	44
2.1.2	General reagents	44
2.1.3	Reagents for cell culture	45
2.2	Instrumentation	45
2.3	Methods	52
2.3.1	Fluorescence quantum yields (Φ_F)	52
2.3.2	Triplet quantum yields (Φ_T) and lifetimes (τ_T)	52
2.3.3	Singlet oxygen (ϕ_Δ) quantum yields	53
2.3.4	Photocatalytic reactions	54
2.3.5	<i>In vitro</i> PDT cell studies	54
2.3.5.1	Cell culture	54

2.3.5.2	Preparation of cells for experiments.....	55
2.3.5.3	Dark cytotoxicity.....	55
2.3.5.4	Light toxicity.....	56
2.3.5.5	Photodynamic therapy (PDT) studies	56
2.4	Synthesis	57
2.4.1	Phthalonitrile synthesis	57
2.4.1.1	3-(2-Benzothiazol-2-yloxy)-phthalonitrile (Scheme 3.1, complex 13)	57
2.4.1.2	3-(Carbazol-2-yloxy)phthalonitrile (Scheme 3.1, complex 15)	58
2.4.1.3	4-(9H-Carbazol-2-yloxy)-phthalonitrile (Scheme 3.1, complex 16)	58
2.4.2	Preparation of phthalocyanines.....	59
2.4.2.1	2,(3)- Tetra (carbazol-2-yloxy)phthalocyaninato zinc (II) (Scheme 3.2, 7a)	59
2.4.2.2	1,(4)- Tetra (carbazol-2-yloxy)phthalocyaninato zinc (II) (Scheme 3.2, 7b)	60
2.4.2.3	1,4-Tetrakis[2-dioxa-8-azaspiro[4.5]dec-8-yl)ethoxy]phthalocyanine tetraiodide (Scheme 3.3, 8)	60
2.4.2.4	1,(4)-Tetra (2-benzothiazol-2-yloxy) phthalocyaninato zinc (II) (Scheme 3.2, complex 9)	61
2.4.3	Synthesis of ZnTAPc (10)-FA conjugate (Scheme 3.4).....	62
2.4.4	Tris {9(10),16(17),23(24)-4-(2-mercaptopyridine)-2-(4-carboxyphenoxy) phthalocyaninato} magnesium (II)-polylysine conjugate (Scheme 3.5)	63
2.4.5	Conjugation of Pcs to NPs or MPs.....	63
2.4.5.1	Synthesis of MPc-ZnO conjugate (Scheme 3.6).	63
2.4.5.2	Synthesis of MPc-AgNPs conjugate (Scheme 3.7).	64
2.4.6	Preparation of electrospun fibers.....	64

2.4.7	Preparation of chitosan-beads.....	65
CHAPTER 3.....		66
SYNTHESIS & CHARACTERIZATION.....		66
PUBLICATIONS		67
3.1	Synthesis and characterization of MPcs and MPcs conjugated to folic acid or polylysine	69
3.1.1	Synthesis and characterization of Pcs (7a, 7b, 8 and 9)	69
3.1.2	Synthesis and characterization of 10-FA	74
3.1.3	Synthesis and characterization of 11-PLL.....	78
3.2	Ground state absorption spectra of MPcs.....	80
3.2.1	Ground state absorption spectra of Pcs (7a, 7b, 8 and 9).....	80
3.2.2	Ground state absorption spectra of 10-FA and 11-PLL	85
3.3	Phthalocyanine-nanoparticle conjugates.....	89
3.3.1	Complex 7a-ZnOMPs conjugates.....	90
3.3.2	Phthalocyanine-AgNPs conjugates.....	94
3.4	Characterization of supports: electrospun fibers and chitosan beads	98
3.4.1	Scanning electron microscopy (SEM).....	99
3.4.2	Flouresence microscopy	102
3.4.3	FTIR spectra	103
3.4.4	TGA/DTA analysis.....	104
3.4.5	Ground state absorption spectra.....	107
3.4.5	X-Ray powder diffraction patterns.....	108
CHAPTER 4.....		111
PHOTOPHYSICOCHEMICAL PROPERTIES.....		111
4.1	Photophysical and photochemical behaviour of MPcs.....	112
4.1.1	Fluorescence spectra, quantum yields and lifetimes of MPcs	112
4.1.2	Triplet quantum yields and lifetimes of MPcs	115

4.1.3	Singlet oxygen quantum yields of MPcs	117
4.2	Photophysicochemical behaviour of 10 -folic acid conjugate	119
4.2.1	Fluorescence spectra, quantum yields (ϕ_F) and lifetimes (τ_F)	119
4.2.2	Triplet quantum yields (Φ_T) and lifetimes	121
4.2.3	Singlet oxygen quantum yields	122
4.3	Photophysicochemical behaviour of MPc-particle conjugates	124
4.3.1	Fluorescence spectra, lifetimes and quantum yields	124
4.3.2	Triplet quantum yields and lifetimes	127
4.3.3	Singlet oxygen quantum yield studies	128
4.4	Photochemical behavior of supports: electrospun fibers and chitosan beads	130
CHAPTER 5		132
PHOTOTRANSFORMATION OF ORGANIC POLLUTANTS		132
5.	Phototransformation of organic pollutants	133
5.1.	Photocatalytic degradation of Rhodamine 6G	134
5.1.1.	UV-Vis spectral changes	134
5.1.2.	Reaction kinetics	137
5.1.2.1	Electrospun fibers	137
5.1.2.2	Reaction kinetics of chitosan beads	144
5.1.3	Chromatographic analysis	149
5.1.4	Catalyst stability and recyclability	151
5.2	Photocatalytic degradation of methyl orange	153
5.2.1	UV-Vis spectral changes	153
5.2.2	Reaction kinetics	155
CHAPTER 6		160
PHOTODYNAMIC THERAPY OF CANCER		160
6.1.	Photodynamic therapy of phthalocyanines: <i>In vitro</i> cell studies	161

6.1.1	PDT of 8 in the presence of AgNPs on melanoma cancer cell line.	161
6.1.2	PDT of 10 and 10-folic acid conjugate on MCF-7 breast cancer cell line	167
6.1.3	PDT of 11 and 11 polylysine conjugate on prostate cancer cell line..	172
CHAPTER 7		174
CONCLUSIONS AND FUTURE PROSPECTS		174
7.1	Conclusions	175
7.2	Future Prospects	176
REFERENCES		178

LIST OF SYMBOLS

α	non-peripheral position
β	peripheral position
ε	molar extinction coefficient
λ	wavelength
τ	lifetime
τ_0	fluorescence radiative lifetime
τ_F	fluorescence lifetime
τ_T	triplet state lifetime
$t_{1/2}$	half-life
Φ	quantum yield
Φ_F	fluorescence quantum yield
Φ_T	triplet state quantum yield
Φ_Δ	singlet oxygen quantum yield
A/abs	absorbance/absorption
F	area under fluorescence emission curve
$\text{O}_2(^1\Delta_g)$	singlet molecular oxygen
S_0	singlet ground state
S_1	singlet excited state
t	time
T_1	triplet excited state
nm	nanometre
cm^{-1}	per centimetre

LIST OF ABBREVIATIONS

Pc	Phthalocyanine
MPc	Metallophthalocyanine
H ₂ Pc	Free base phthalocyanine
NPs	Nanoparticles
AgNPs	Silver nanoparticles
ZnOMPs	Zinc oxide microparticles
DMSO-d ₆	Deuterated dimethylsulfoxide
D ₂ O	Deuterated water
HOMO	Highest occupied molecular orbital
LUMO	Lowest unoccupied molecular orbital
CB	Conduction band
VB	Valence band
a.u	Arbitrary units
DPBF	Diphenylisobenzofuran
CT	Charge-transfer transitions
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMSO	Dimethylsulfoxide
ET	Energy transfer
F	Fluorescence
IC	Internal conversion
IR	Infrared
ISC	Intersystem crossing

LMCT	Ligand-to-metal charge transfer
PDT	Photodynamic therapy
Ph	Phosphorescence
ROS	Reactive Oxygen Species
TCSPC	Time-correlated single photon counting
TEM	Transmission electron microscope
SEM	Scanning electron microscope
PS	Polystyrene
¹ H NMR	Proton nuclear magnetic resonance
TGA	Thermogravimetric analysis
TRES	Time-resolved emission spectra
UV/Vis	Ultraviolet/visible
XRD	X-ray diffractometer
Rh 6G	Rhodamine 6G
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
ADMA	Tetrasodium,α-(anthracene-9,10-diyl)dimethylmalonate
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

LIST OF FIGURES

<i>Figure</i>	<i>Description</i>	<i>Page</i>
1.1	Modified schematic diagram showing different strategies for nanoparticle surface passivation: electrostatic repulsion (a) and steric hindrance (b) [21].	4
1.2	Formation of reactive oxygen species by the photosensitization of ZnO upon exciting the Pc with the UV light. VB= valence band, CB= conduction band, HOMO= highest occupied molecular orbital, LUMO= lowest occupied molecular orbital.	6
1.3	Phthalocyanine ring showing potential sites for substitution (numbering traditionally used for Pc nomenclature).	7
1.4	Non-peripherally and peripherally substituted H ₂ and Zn phthalocyanines synthesized and reported in this work.	13
1.5	Typical ground state electronic absorption spectra of free-base (a) and metallated phthalocyanine (b).	14
1.6	Electronic energy orbital diagram showing the origin of the two major absorption bands in phthalocyanines.	15
1.7	A modified model showing exciton splitting of the Q-band between two MPc molecules on aggregating. The solid vertical arrows represent allowed transitions, the dashed vertical arrow represents forbidden transition.	17

1.8	A modified Jablonski diagram showing the transition between the singlet ground state (S_0) and electronic excited states (S_1 and T_1). A = absorption, F= fluorescence, VR = vibrational relaxation, IC = internal conversion, ISC = intersystem crossing, P = phosphorescence. S_1 = singlet excited state and T_1 = triplet excited state. ET= energy transfer.	17
1.9	Some selected first and second generation photosensitizer in clinical trials.	27
1.10	Chemical structure of folic acid.	29
1.11	Internalization of folic acid-drug conjugate by endocytosis [122].	30
1.12	Typical diagrammatic set-up of electrospinning apparatus	36
1.13	Chemical structure of Rhodamine 6G (A) and methyl orange (B)	39
2.1	Schematic diagram for a laser flash photolysis setup. PMT = Photomultiplier tube	49
2.2	Schematic diagram of photo-irradiation setup.	50
2.3	Typical Electrospinning set-up.	50
3.1	Mass spectrum of complex 7a .	74
3.2	FTIR spectra of 10-FA (i), folic acid (ii) and 10 (iii).	76

3.3	TLC plates of the conjugate prior to the separation (a) and after the purification (b) by using size exclusion. TLC plates are viewed under UV light.	80
3.4	Absorption spectra of 7a and 7b in DMSO.	81
3.5	Absorption spectra of 7b (A), 7a (B) and 9 (C) in different organic solvents	83
3.6	Absorption spectra of complex 8 in DMF and in water. The figure also shows the breaking of aggregates by adding the surfactant SDS.	85
3.7	Absorption spectra of 10 +FA mixture (a), 10 -FA covalently linked (b), folic acid (c) and 10 (d) in DMSO.	87
3.8	Ground state absorption spectra of 10 -FA in water (a), 10 -FA in DMSO (b) and folic acid in water	88
3.9	Absorption spectra of 11 -polylysine in water as a solvent.	88
3.10	SEM image of ZnOMPs and the corresponding particle size distribution.	90
3.11	IR spectrum of the conjugate, 7a , ZnOMPs and the conjugate of 7a -ZnOMPs	92
3.12	XRD pattern of ZnOMPs, complex 7a and 7a -ZnOMPs conjugate.	93

3.13	UV-Vis spectra of 7a and when covalently conjugated to ZnOMPs. Spectra were acquired in DMSO as a solvent.	94
3.14	TEM images, with the corresponding size distribution of AgNPs (A) and 7a -AgNPs conjugate (B) in DMSO as a solvent.	96
3.15	UV-Vis spectra of 7a and 10 on conjugating to AgNPs in DMSO as a solvent.	97
3.16	SEM micrographs of PS (A), 7a /PS (B), 7a -ZnOMPs/PS (C) and ZnOMPs/PS (D). EDX plots corresponding to the 7a -ZnOMPs/PS fiber with (E) and without (F) ZnOMPs.	101
3.17	SEM images of 10 -AgNPc/chitosan (A) after drying at 60 °C with the corresponding EDX (B).	102
3.18	Fluorescence micrographs of ZnOMPs/PS (a), 7a /PS (b) and 7a -ZnOMPs/PS (c).	103
3.19	FTIR spectra of chitosan (A), 10 (B) and 10 /chitosan (C) beads dried at 60 °C.	104
3.20	TGA (A) and DTA (B) thermograms of PS and PS functionalized nanofibers.	106
3.21	Thermograms of 10 , chitosan beads and chitosan beads functionalized with 10 and 10 -AgNPs.	107
3.22	Absorption spectra of 7a /PS, 7a -ZnOMPs/PS and ZnOMPs/PS dissolved in toluene.	108

3.23	XRD pattern of PS, ZnOMPs/PS, 7a /PS and 7a -ZnOMPs/PS.	109
3.24	XRD diffraction patterns of pristine chitosan (A), 10 /chitosan (B) and 10 -AgNPs/chitosan (C) beads.	110
4.1	Absorption (i), excitation (ii) and emission (iii) spectra of complex 7a (A), 7b (B), 8 (C) and 9 (D) in DMSO as a solvent.	113
4.2	Fluorescence decay curves of complex 8 and 7b in DMSO. Excitation wavelength, 680 nm.	114
4.3	Transient curve of complex 7b , along with 7b triplet decay curve (Excitation = 540 nm).	116
4.4	A typical spectra for the determination of singlet oxygen quantum yield in DMSO using complex 7a as an example.	119
4.5	Emission (i), absorption (ii) and excitation (iii) spectra of 10 -FA conjugate in DMSO. Excitation wavelength = 640 nm.	120
4.6	Time resolved emission spectra (TRES) of 10 -FA in DMSO. Long lifetime at 2.27 ns (i) and a short lifetime of 1.45 ns (ii).	121
4.7	Bi-exponential triplet decay curve of 10 -FA in DMSO.	122
4.8	A typical spectrum for the determination of singlet oxygen quantum yield. The spectra shows the degradation of DPBF in the presence on 10 -FA.	123
4.9	Absorption, excitation and emissions spectra of complex 7a -ZnOMPs (A) and 7a -AgNPs conjugate in DMSO (B).	125

4.10	Time correlated single photon counting (TCSPC) fluorescence decay curves of 7b (i), and 7b -AgNPs (ii) in DMSO.	126
4.11	Time resolved emission spectra (TRES) of 7b -AgNPs in DMSO. Emission at ~680 nm (a) is due to 4.89 ns and emission at 700 nm (b) is from 2.65 ns.	127
4.12	Degradation profile of ADMA ($\sim 3.33 \times 10^{-5}$ M) using 7b -AgNPs/PS (A) and 10 -AgNPs/chitosan beads (B) in water (1 min interval).	131
5.1	Spectral changes observed during the phototransformation of Rh 6G, with 7a /PS as a catalyst under pH 2 conditions. Time interval = 10 min.	135
5.2	Decrease in Rhodamine 6G fluorescence with illumination in the presence of 7a - as a catalysts. a) Rhodamine 6G at t=0, and b) shows Rhodamine 6G after 60 min of irradiation.	136
5.3	First order kinetics plots for the photodegradation of Rh 6G under pH 9.2 (A) and pH 2 conditions (B), with 7a -AgNPs (i), 7a -ZnOMPs (ii) and 7a (iii) as photocatalysts.	139
5.4	Plots of the reciprocal of the initial reaction rate versus the reciprocal of the initial concentration for transformation of Rhodamine 6G. pH 9.2.	143
5.5	First order kinetics plots for the degradation of Rhodamine 6G at different concentrations with 10 /chitosan (A) and 10 -AgNPs/chitosan (B) beads. Initial concentration of	145

Rhodamine 6G = 2.18×10^{-6} (i) 9.69×10^{-6} (ii) and 20.1×10^{-6} M (iii).

- | | | |
|------|---|-----|
| 5.6 | Plots of the reciprocal of the initial reaction rate versus the reciprocal of the initial concentration for the degradation of Rhodamine 6G with 10 -AgNPs/chitosan (i) and 10 /chitosan (ii) beads as photocatalysts. | 149 |
| 5.7 | HPLC chromatograms (A) of phototransformation products obtained at $t = 0$ and after 40 min irradiation time, and the UV-Vis spectra (B) of the peaks corresponding to 8, 11 and 12 min in the HPLC spectrum obtained in $t = 40$ min. The photocatalysts employed was 7a /PS at pH 9.2. | 150 |
| 5.8 | Percentage Rh 6G removal, under pH 2 conditions, in the presence of the unmodified photocatalysts (7a /PS). | 152 |
| 5.9 | UV-Vis spectral changes of an aqueous solution of methyl orange (pH=9.2). Initial conc. of methyl orange = 2.31×10^{-5} M) containing 20 mg of 7b -AgNPs/PS. | 154 |
| 5.10 | First order kinetics plots for the degradation of methyl orange at different concentrations with 7b /PS. Studies were done in water at pH 9.2. | 156 |
| 5.11 | Plots of the reciprocal on initial reaction rate versus the reciprocal of the initial concentration for degradation of methyl orange. Studies were done in water at pH 9.2. | 158 |

6.1	Dark cytotoxicity of complex 8 and complex 8 + 5% AgNPs. Data represents the mean values from three independent experiments. Error bars are standard deviations.	163
6.2	Laser dose toxicity on melanoma cancer cell lines. The data represent the mean values from three independent experiments. Error bars are standard deviations.	164
6.3	Images of melanoma cancer cells after incubating for 24 hr with 8 (A) and 8 +AgNPs (B) in the dark. C and D show cells incubated for 24 hr. with 8 and 8 + 5% AgNPs, respectively, and irradiated with 2.5 J/cm ² .	165
6.4	Photodynamic cytotoxicity of complex 8 and complex 8 + 5% AgNPs. Irradiation dose = 2.5 J/cm ² . Data represent the mean values from three independent experiments. Error bars are standard deviations.	167
6.5	Percentage survival of MCF-7 cells when incubated with different concentrations of 10 and 10 -FA for 24 hours under conditions detailed in the experimental section.	168
6.6	Photodynamic inactivation of MCF-7 cells with 10 (A) and 10 -FA (B) illuminated with power densities of 22.9, 45.8 and 91.6 J/cm ²	170
6.7	Micrographs of MCF-7 cells in the presence of 160 µM of 10 -FA before (A) and after irradiation (B) with a power density of 91.6 J/cm ² .	171
6.8	Percentage cell survival of irradiated PC-3 cells with 11 and 11 -polylysine conjugates	173

LIST OF SCHEMES

<i>Scheme</i>	<i>Description</i>	<i>Page</i>
1.1	Synthesis of unsubstituted metallophthalocyanines (MPcs) from different starting materials.	9
1.2	Substitution reaction of 3 or 4-nitro phthalonitrile with alkoxy groups to produce a peripheral or non-peripheral substituted phthalocyanine.	11
2.1	Synthetic routes to the phthalonitriles reported in this work.	70
3.2	Synthesis of 7a , 7b and 9 .	72
3.3	Synthesis of complex 8 .	73
3.4	Conjugation of FA to 10 via the condensation reaction between activated folic acid and 10 .	75
3.5	Conjugation of 11 and polylysine by the activation of 11 with DCC, NHS coupling agents.	79
3.6	Synthesis and Interaction of ZnOMPs with 7a . The carboxylic acids of the capping agents are activated to form the amide bond with the secondary amine of 7a .	91
3.7	Schematic representation of the interaction between silver nanoparticles and 10 .	95

5.1	Deethylation of Rhodamine 6G by singlet oxygen produced upon exciting the phthalocyanine.	136
5.2	Type II (A) and type I (B) photosensitization of Rhodamine 6G with immobilized 10 and 10 -AgNPs. Sub represent substrate which is Rhodamine 6G.	147

LIST OF TABLES

<i>Table</i>	<i>Description</i>	<i>Page</i>
1.1	Phthalocyanines conjugated to various nanoparticles.	24
1.2	Phthalocyanines conjugated to Folic acid and polylysine.	32
1.3	Phthalocyanines-nanoparticle conjugates supported on fibers or chitosan beads for dye removal.	38
3.1	Ground state absorption spectra of 7a , 7b , 8 and 9 in different solvents.	84
3.2	Summary of nano or microparticles conjugated to the reported Pcs.	90
3.3	Summary of complexes and conjugates immobilized on PS fibers or chitosan beads.	99
4.1	Photophysics and photochemical behaviour of complexes in DMSO.	118
4.2	Photophysicochemical parameters of 10 and its conjugate with folic acid in DMSO.	123
4.3	Photophysicochemical parameters of complexes on conjugating to nanoparticles. Data was acquired in DMSO.	129

5.1	Summary of catalysts used in dye degradation.	134
5.2	Kinetic data of transformation of Rh 6G using the unmodified and hybrid photocatalyst. Excitation wavelength = 670 nm, Conc= 5.13×10^{-6} .	140
5.3	Kinetic data for PS fibers with various concentrations of Rhodamine 6G (pH=9.2).	141
5.4	Langmuir-Hinshelwood parameters for the photocatalysis of Rh 6G with various catalysts.	143
5.5	Kinetic data for PS fibers with various concentrations of Rhodamine 6G (pH=9.2).	146
5.6	Kinetic data for PS fibers with various concentrations of methyl orange (pH=9.2).	157
5.7	Langmuir-Hinshelwood parameters for the photocatalysis of methyl orange Studies were done in water at pH 9.2.	159

CHAPTER 1

INTRODUCTION

Preface: This thesis focuses on the influence of Ag and ZnO nanoparticles on phthalocyanines' phototransformation of organic pollutants and *in vitro* photodynamic therapy. Firstly, the selected nanoparticles will be discussed, this is then followed by a brief description of phthalocyanines' properties, and the general impact of nanoparticles on these properties. The introduction will also give the reader a brief insight into the selectivity of phthalocyanines, and the methods employed in this work to improve the cancer targeting ability of the selected phthalocyanines. General methods of immobilizing phthalocyanines for heterogeneous catalysis are introduced and those that were employed in this study are further discussed.

1.1 Nanoparticles

The IUPAC (International Union of Pure and Applied Chemistry) defines nanoparticles (NPs) as particles with sizes from 1-100 nm [1]. At this size, these particles exhibit novel size related physicochemical properties that differ greatly from their bulk material counterparts [1,2]. The properties of NPs have been exploited in a wide range of applications such as in medicine, cosmetics, renewable energy, environmental remediation and biomedical devices [1,3]. Amongst the different nanoparticles reported to-date, silver and zinc oxide nanoparticles are the most studied, and are employed in this work to enhance the photocatalytic and photosensitizing behaviour of phthalocyanines.

1.1.1 Silver nanoparticles (AgNPs)

Silver nanoparticles are normally characterized by a sharp, localized surface resonance plasmonic (LSPR) band at ~ 390 nm, which depends on the size and shape of nanoparticle [4]. Silver nanoparticles have found applications in various fields, in particular, the antimicrobial and anticancer properties of silver nanoparticles have made them popular in biological applications. As antibacterial agents, AgNPs have been used in disinfecting medical devices and home appliances. They have also been incorporated into textile fabrics for the purpose of wound dressing and water purification [5-8]. Furthermore, the use of AgNPs as electrochemical sensors resulted in faster response times and lower detection limits [9].

Catalysis is improved by the presence of silver nanoparticles, e.g. Kohler *et al.* showed that bleaching of organic dyes by the application of potassium peroxodisulphate in aqueous solution, at room temperature, was strongly enhanced in the presence of silver nanoparticles [10]. Reports indicate that exciting the nanoparticle at the plasmonic resonances induces the production of reactive oxygen species (ROS) [11-14]. Anticancer properties of silver nanoparticles are due to the generated ROS which oxidize the organelles of the tumor cells [15,16]. The use of nanoparticles in conjunction with other therapeutic drugs is reported to enhance the drugs' efficacy. This has been attributed to the increased production of ROS and the enhanced permeability and retention (EPR) effect which will be discussed later [17-19]. In this work, AgNPs are employed to enhance the photodynamic therapy and photocatalytic activities of phthalocyanines (Pcs) through EPR and enhanced singlet oxygen production (the latter is a result of the heavy atom effect that will be discussed later).

The synthesis of silver nanoparticles can be divided into chemical and physical approaches [12,20]. The chemical method is based on the reduction of a metal salt ion, with strong reducing agents, under reaction conditions that favour the formation of metal clusters [12]. Physical methods entail the synthesis of silver nanoparticles by evaporation-condensation steps carried out in a tube furnace at atmospheric pressure [12,20]. Another reported physical synthetic method for silver nanoparticles is the laser ablation of metallic bulk materials in solution [20]. Although physical methods are efficient, they have several drawbacks when compared to the frequently used chemical method. The latter is preferred as it provides ease of manipulation of size

and shape. With the chemical method, synthetic conditions such as: temperature, reactant concentration and type of passivation agents used, can be easily optimized so as to obtain the desired size and shape of the nanoparticles. Different capping molecules are chosen based on the desired shape and particle size. A capping ligand controls the size by inducing electrostatic repulsion, e.g. capping nanoparticles with ionic citric acid. It can also encourage steric hindrance, e.g. the use of non-polar long chained organic molecules such as tri-*octyl*-phosphine oxide (TOPO) as shown in **Figure 1.1** [21]. In this work, spherical, citric acid capped silver nanoparticles were synthesised using a well reported chemical method [4].

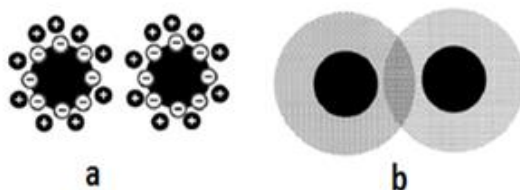


Figure 1.1: Modified schematic diagram showing different strategies for nanoparticle surface passivation: electrostatic repulsion (a) and steric hindrance (b) [21].

1.1.2 Zinc Oxide (ZnO) nanoparticles

Zinc oxide is an important member of the II-IV group semiconductors, with a wide band gap of 3.20 eV and a large exciton binding energy of 60 meV [22]. ZnO nanoparticles have found applications as sensors, in photocatalysis, in optics, as actuators and in photovoltaic cells [23-29]. ZnO nanoparticles have shown intriguing antimicrobial and anticancer properties. Similar to silver nanoparticles, reactive

oxygen species are also produced upon exciting ZnO nanoparticles with the appropriate wavelength of light [30-33].

ZnO readily forms different shapes of the nano or micro sized particles [22]. A number of methods have been reported for the synthesis of ZnO nanoparticles, however, the sol-gel method, pioneered by Spanhel and Anderson, is the simplest and commonly used approach [34]. Other methods for the preparation of ZnO nanoparticles include the double jet precipitation method [35], flow through supercritical water method (FT-SCW) established by Sue *et al.* [36], and the reverse micelle method [37]. A non-hydrolytic single source precursor for the synthesis of different metal oxides was reported by Rockengerger *et al.*, where they used metal Cupferron complexes [38]. Of all methods reported, the wet chemical (hydrothermal/ solvothermal) approach is the easiest and the most inexpensive; hence, it is employed in this work.

ZnO exhibit interesting photocatalytic properties in aqueous media, however, the downside of using ZnO as a photocatalyst is the limitation of the exciting light to the UV region of the spectrum. UV radiation reaching the earth is only 4-6% of the solar irradiation, 45 % constitutes visible light [39]. Hence, better methods for harnessing the UV-Vis radiation have been explored. The photosensitization of ZnO with a dye which absorbs in the visible region has proven to be effective and convenient [40]. Two processes occur during the excitation of the phthalocyanine in the presence of ZnO (**Figure 1.2**): upon excitation with visible light (1), the electron hole pair is formed in the HOMO and LUMO of the phthalocyanine. The electron in the LUMO is then

injected into the conduction band of ZnO, followed by generation of the dye cation (4). The charged species undergo several reactions forming ROS (3 and 5). The charge injection process from a dye to a semiconductor has been typically applied in photoelectrochemical cells [41]. A second possible mechanism involves the intersystem crossing (ISC) of the excited dye to the forbidden triplet state, with subsequent reactions resulting in the formation of ROS (2). Thus, phthalocyanines can act as co-catalyst (with ZnO), by forming reactive oxygen species such as singlet oxygen (2) and others. Therefore, in this work we combine phthalocyanine with ZnO for improved photocatalytic behavior, as a result of an increase in ROS formation and the heavy atom effect induced by the presence of ZnO NPs.

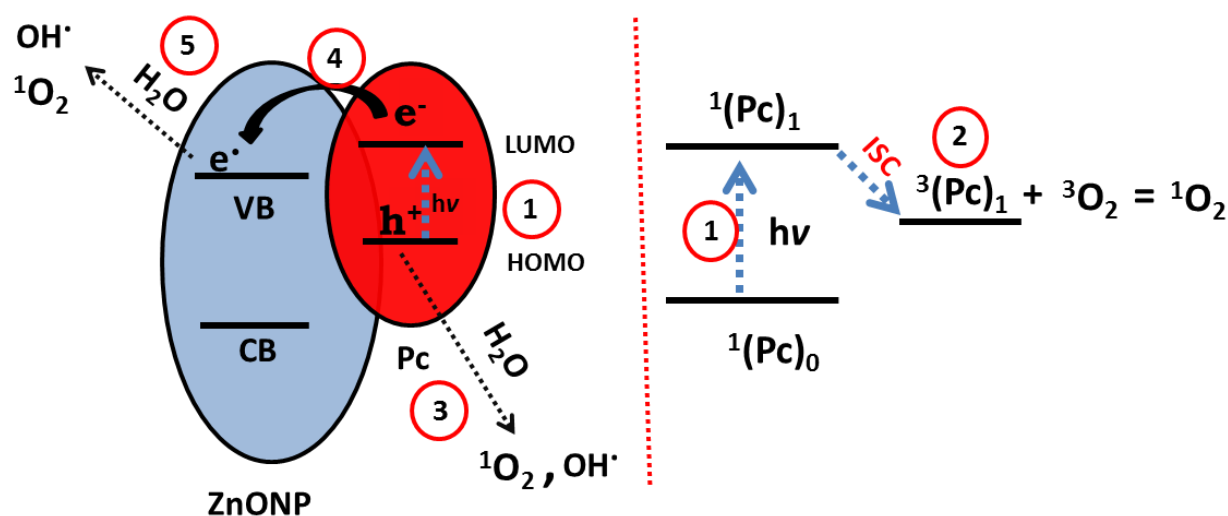


Figure 1.2: Formation of reactive oxygen species by the photosensitization of ZnO upon exciting the Pc with the UV light. VB= valence band, CB= conduction band, HOMO= highest occupied molecular orbital, LUMO= lowest occupied molecular orbital.

1.2 Background on Phthalocyanines

1.2.1 Structure, general synthesis and applications

Phthalocyanines are 18 π electron macrocyclic compounds (**Figure 1.3**) that possess remarkable physical and chemical properties. Their conductivity, bright colours, chemical and thermal stability have made them desirable for many applications. Phthalocyanines (Pcs) have traditionally been used as dyes and pigments [42,43]. Phthalocyanines continue to find use in contemporary and emerging technologies such as sensing [44-46], photodynamic therapy [47-49], non-linear optics [50], electrochemical sensors [51], photovoltaic energy conversion [52] and others.

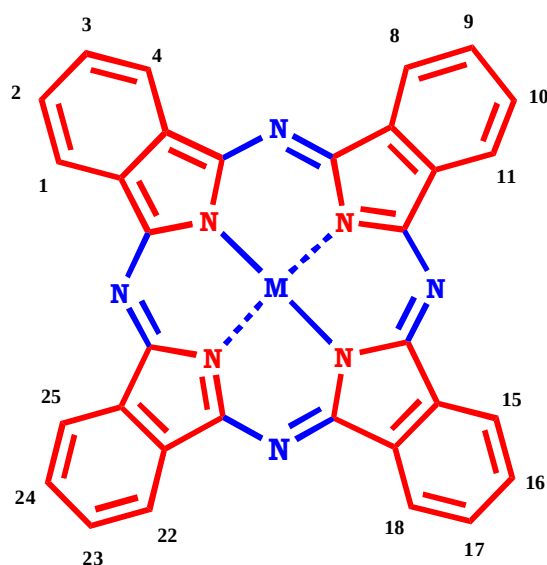
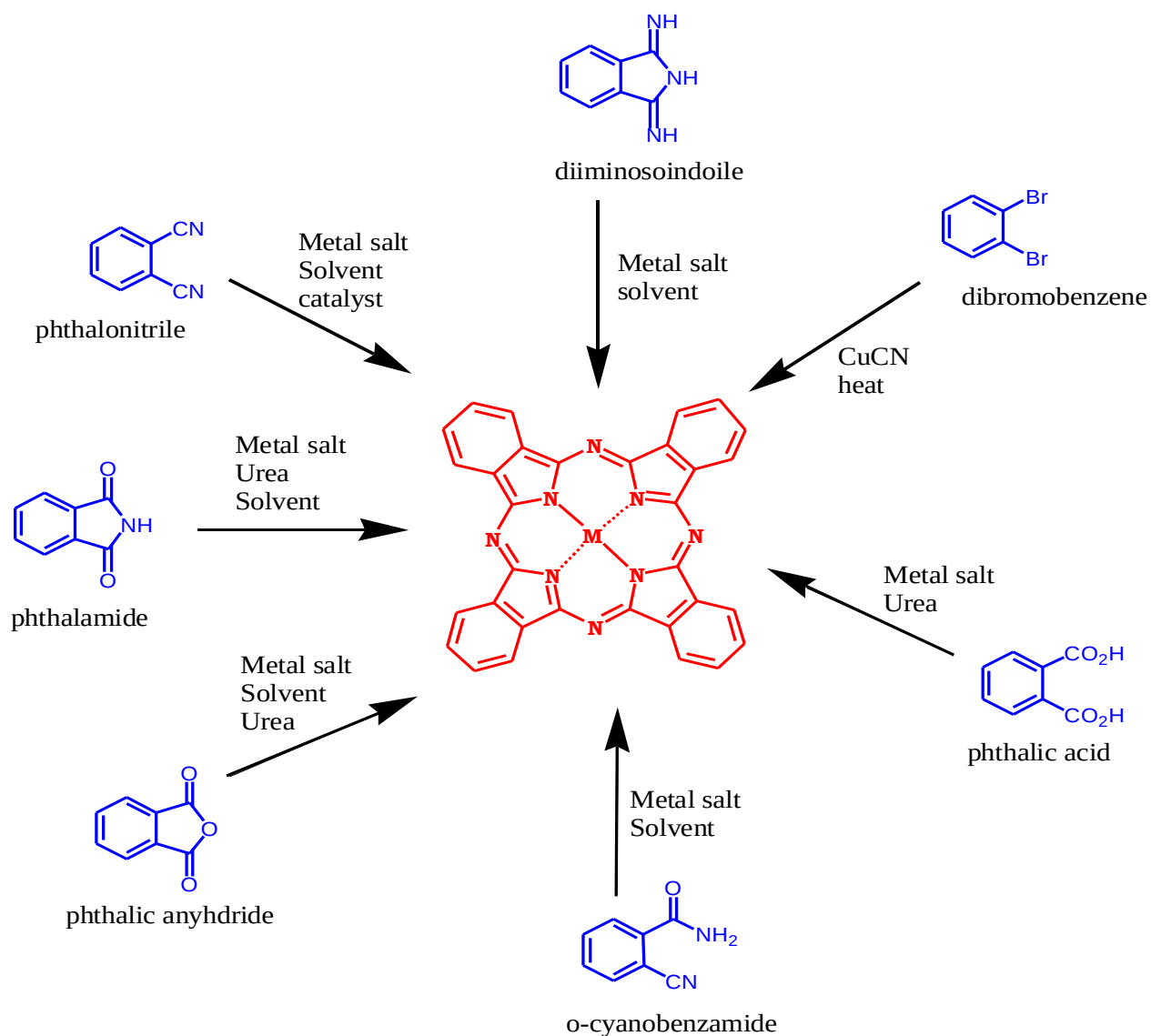


Figure 1.3: Phthalocyanine ring showing potential sites for substitution (numbering traditionally used in Pc nomenclature).

Phthalocyanines are mainly composed of 4-isoindole groups linked by four nitrogen atoms (**Figure 1.3**). The phthalocyanine ring is numbered following the IUPAC

nomenclature of tetrapyrroles as shown in **Figure 1.3** [53]. When a phthalocyanine is coordinated to a metal, it is referred to as a metallophthalocyanine (MPc) [43]. Thermal, physical, chemical and electrochemical properties of these macrocycles are key to their applications, and the properties can be tailored by the careful selection of the central metal, type and position (peripheral and non-peripheral) of the substituents on the MPc ring [54-56].

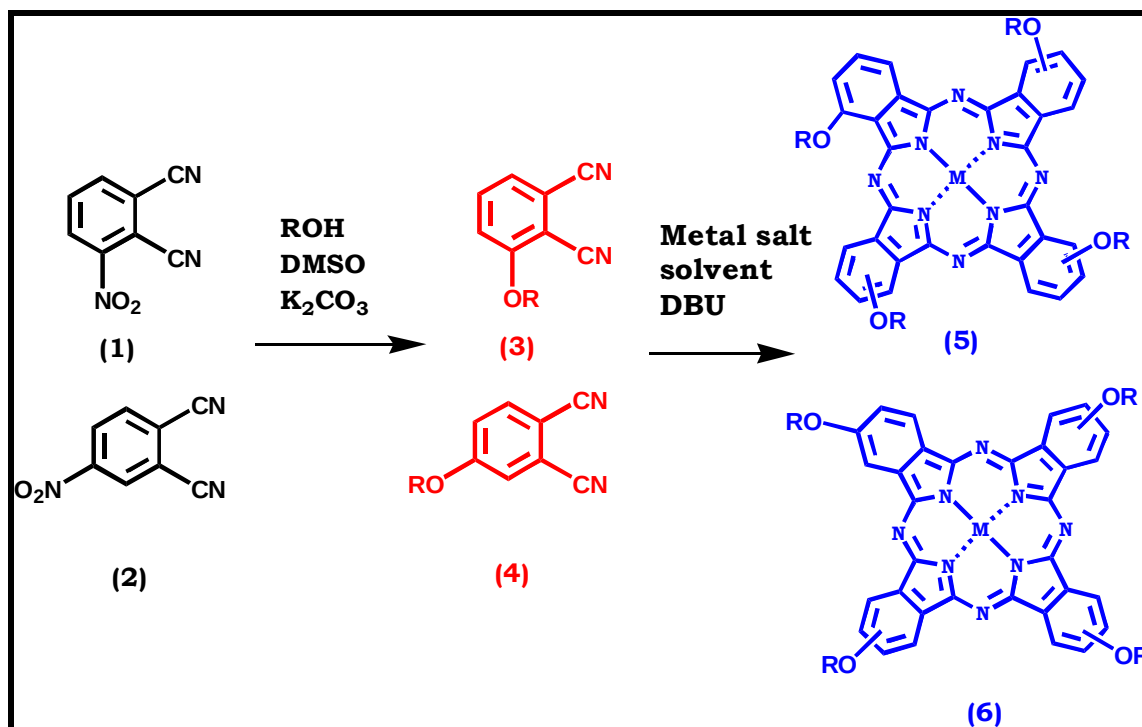
The synthesis of phthalocyanines is generally achieved by the cyclotetramerization of a phthalonitrile precursor in the presence of a metal salt (for MPcs), a solvent and a catalyst [42,43]. Other starting materials such as: phthalamide, phthalic acid anhydride, *o*-cyanobenzamide, phthalic acids, *o*-dibromobenzene and other derivatives can be used in the synthesis of phthalocyanines (**Scheme 1.1**) However, the method employing phthalonitrile precursors is much more popular due to ease of purification and high yields of the desired product [57].



Scheme 1.1: Synthesis of unsubstituted metallophthalocyanines (MPcs) from different starting materials.

1.2.2 Synthesis of peripherally (β) and non-peripherally (α) substituted phthalocyanines

Phthalocyanines are insoluble in a number of organic solvents, this is due to the extensive π conjugation of the ring [43]. The problem of insolubility can be reduced by functionalizing the isoindole group of the ring with different substituents at the peripheral or non-peripheral positions [42,43]. The formation of tetrasubstituted phthalocyanine may begin with the phthalonitrile synthesis; 3 or 4-nitro phthalonitriles (**1** and **2**, respectively in **Scheme 1.2**) which are commercially available, but can also be easily prepared by well documented methods [58,59]. Functionalization of the phthalonitriles with different ligands (**3** and **4**, **Scheme 1.2**) can be attained by nucleophilic reaction with a good electrophile such as an alkoxy or thiol- moiety [60]. The general procedure for tetra substituted peripheral (**5**) and non-peripheral (**6**) phthalocyanines is highlighted in **Scheme 1.2**.



Scheme 1.2: Substitution reaction of 3 or 4-nitro phthalonitrile with alkoxy groups to produce a peripheral or non-peripheral substituted phthalocyanine.

When mono-functionalized phthalonitriles are used, tetrasubstituted MPcs with constitutional isomers are obtained (5 and 6 in **Scheme 1.2**). For peripherally substituted phthalocyanines, the isomers have been identified to be present in the following quantities: 12.5% (C_{4h}), 12.5 % (D_{2h}), 25.0% (D_{2h}) and 50 % (C_s) [61,62]. The allocation of isomers in the non-peripheral substituted phthalocyanine depends on the central metal ion and the structure of the substituent. Isolation to give a single isomer can be achieved through the use of specially designed high performance liquid chromatography (HPLC) columns for separation [63]. The process of isomer separation is time consuming, and it has been reported that the presence of different isomers actually help with the solubility of phthalocyanine. Therefore, in this work, the separation of the constitutional isomers was omitted.

1.2.3 Phthalocyanines synthesized in this work

Phthalocyanines synthesized in this work are shown in **Figure 1.4**. Different substituents were chosen because of the various advantages they offer. The main purpose was to induce solubility in different solvents and to also improve the photophysical and photochemical parameters of the phthalocyanine. Carbazole (7), ketal (8) and benzothiazole (9) ligands were used as substituents in this work (**Figure 1.4**). These substituents were selected since they have shown anticancer properties and can produce singlet oxygen on their own [64-66]. The combination of a benzothiazole, ketal or carbazole skeleton with the phthalocyanine is aimed at enhancing the photosensitizer's activity due to a synergistic effect. The metal centre used is the biocompatible Zn metal ion. Zinc as a central metal has shown improved photophysical properties for the phthalocyanines due to the heavy atom effect which encourages intersystem to the triplet state. A metal free phthalocyanine was also prepared for photodynamic therapy of cancer [67,68].

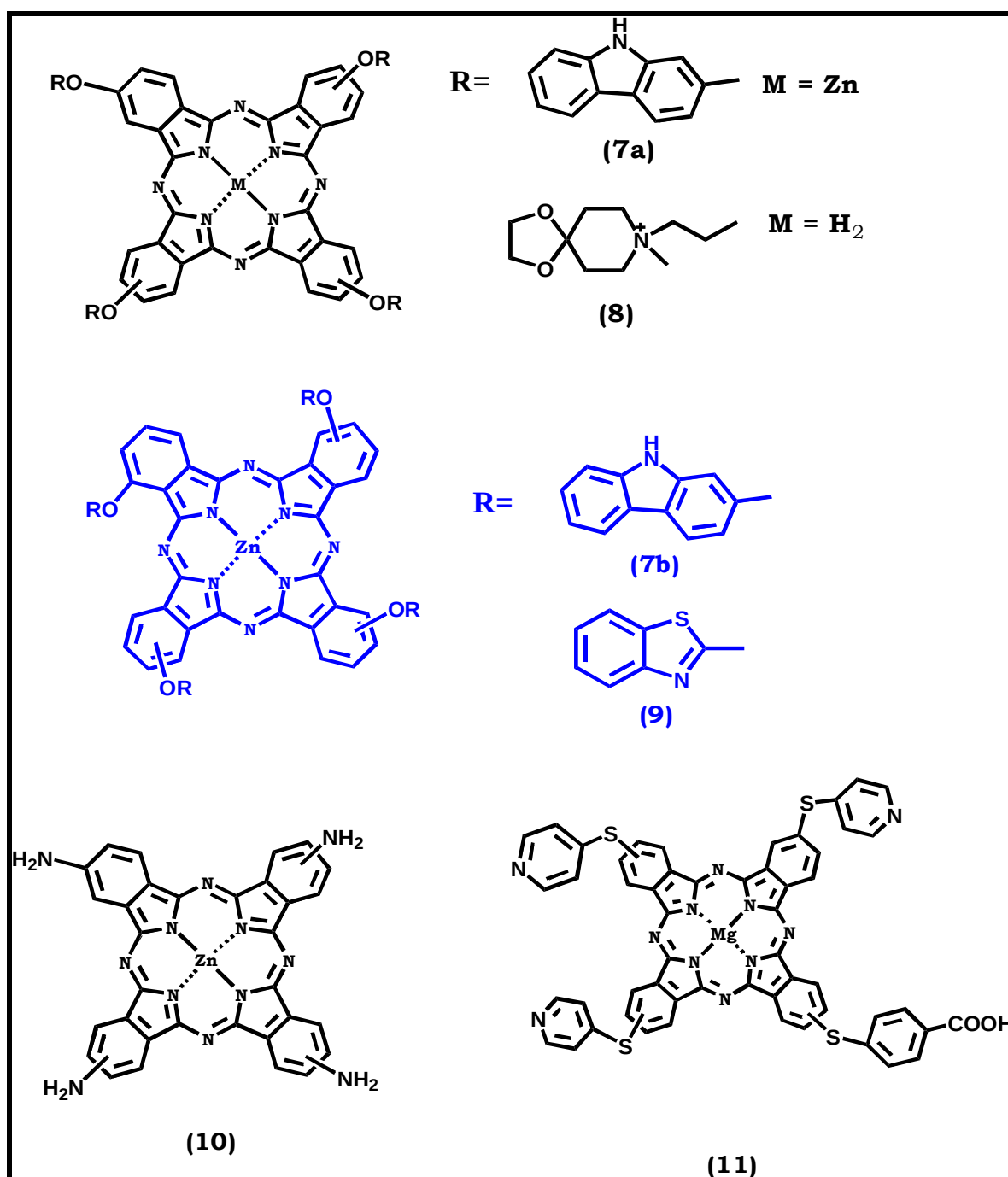


Figure 1.4: Non-peripherally and peripherally substituted H₂ and Zn phthalocyanines synthesized and reported in this work.

1.2.4 Origins of spectra in phthalocyanines

Phthalocyanines are characterized by a strong, isolated Q band in the red region of the UV/Vis spectrum, while the less intense B band is found at higher energies (**Figure 1.5**). The Q band is characterized by a high molar absorptivity ($\epsilon = 10^5 \text{ M}^{-1}\text{cm}^{-1}$) [69], and it is accompanied by a series of vibrational bands. The B band is broad due to the super-positioning of B_1 and B_2 [70].

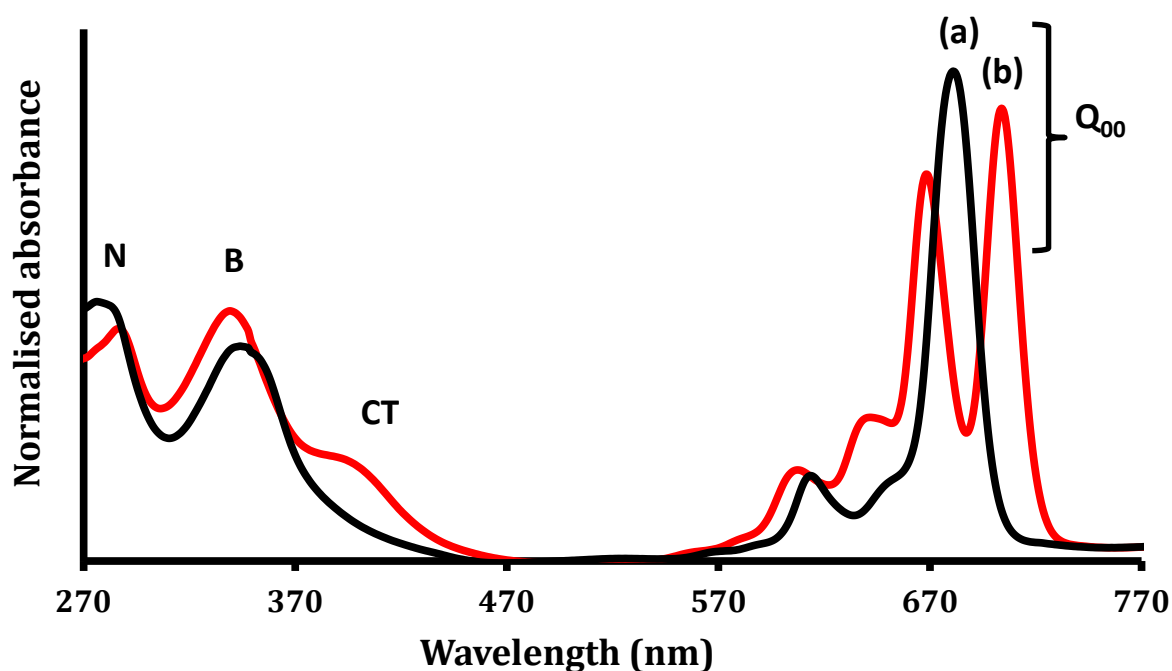


Figure 1.5: Typical ground state electronic absorption spectra of metallated (a) and free-base phthalocyanine (b).

Phthalocyanines are known to be the synthetic analogues of porphyrins, therefore, the Gouterman's four orbital model has been used to explain the spectral evolution of phthalocyanines [71]. According to this model, four frontier orbitals are considered: the HOMO-1, HOMO, LUMO and LUMO+1 orbitals. The first two are occupied, while

the latter are unoccupied and degenerate. The Q and B bands arise from the $\pi - \pi^*$ transitions and are explained in terms of the linear combination of transitions from a_{1u} and a_{2u} HOMO orbitals to the doubly degenerate LUMO orbitals (**Figure 1.6**) [71]. This behaviour is usually observed in metallated phthalocyanines that have D_{4h} symmetry. The absence of a metal ion reduces the symmetry of the Pc to D_{2h} . A decrease in symmetry is associated with an increase in the number of allowed electronic transitions within the molecule [71]. Therefore, the electronic spectrum of a free base Pc, with D_{2h} symmetry, is characterised by a split Q band (**Figure 1.5**), as a result of electronic transition from a_{1u} to the non-degenerate e_g orbital of the LUMO (**Figure 1.6**). Thus, if the symmetry of a metallophthalocyanine is altered from D_{4h} , changes in the spectral features are observed.

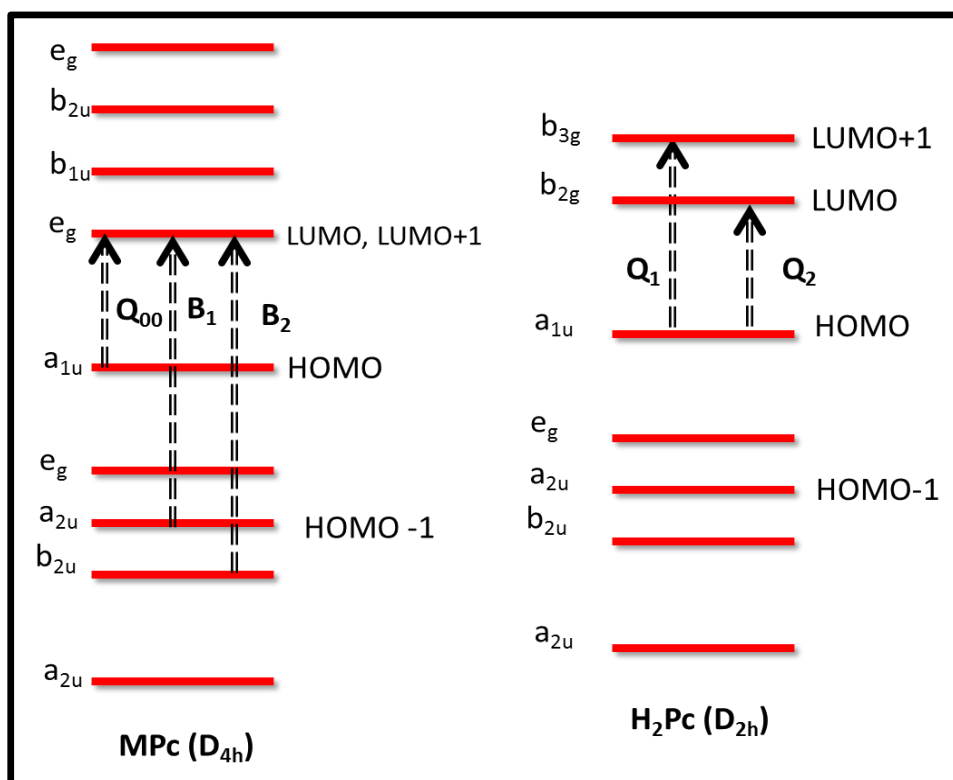


Figure 1.6: Electronic energy orbital diagram showing the origin of the two major absorption bands in phthalocyanines.

1.2.5 Aggregation in phthalocyanines

Phthalocyanine aggregation is referred to as the coplanar association of Pc rings progressing from monomer to dimer and higher-order complexes; this is often driven by π - π interactions between the Pc rings or hydrophobic interactions in polar solvents that arise as a function of their lipophilicity [72-74]. Hence, aggregation is more highly pronounced in polar solvents than in non-polar solvents. MPc aggregation is recognized by broadening and/or splitting of the Q band and a hypsochromic (blue) or bathochromic (red) shift [75] indicating the presence of additional electronic levels.

According to the exciton coupling theory, when two adjacent MPc complexes interact, there is an appearance of degenerate states leading to broadening of spectra indicating the formation of aggregates [76]. The degenerate states arise from the splitting of the e_g excited states, **Figure 1.7**. This results in symmetry allowed and forbidden transitions. The forbidden transitions will still occur to a small extent resulting in the spectral behaviors which are observed as either broadening of the spectra, or complete split and shifts in the Q band.

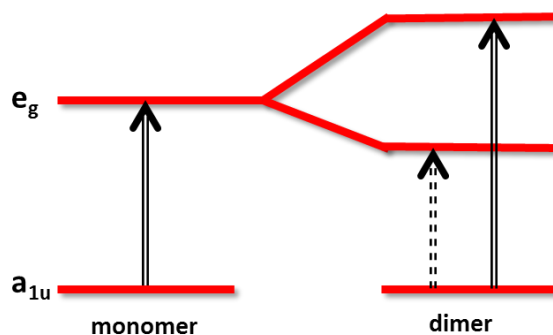


Figure 1.7: A modified model showing exciton splitting of the Q-band between two MPc molecules on aggregating. The solid vertical arrows represent allowed transitions, the dashed vertical arrow represents forbidden transition.

1.2.6 Photophysics and photochemistry of phthalocyanines

The series of elementary processes that occur upon the interaction of light with phthalocyanines can be explained with a Jablonski diagram (**Figure 1.8**) [77-80].

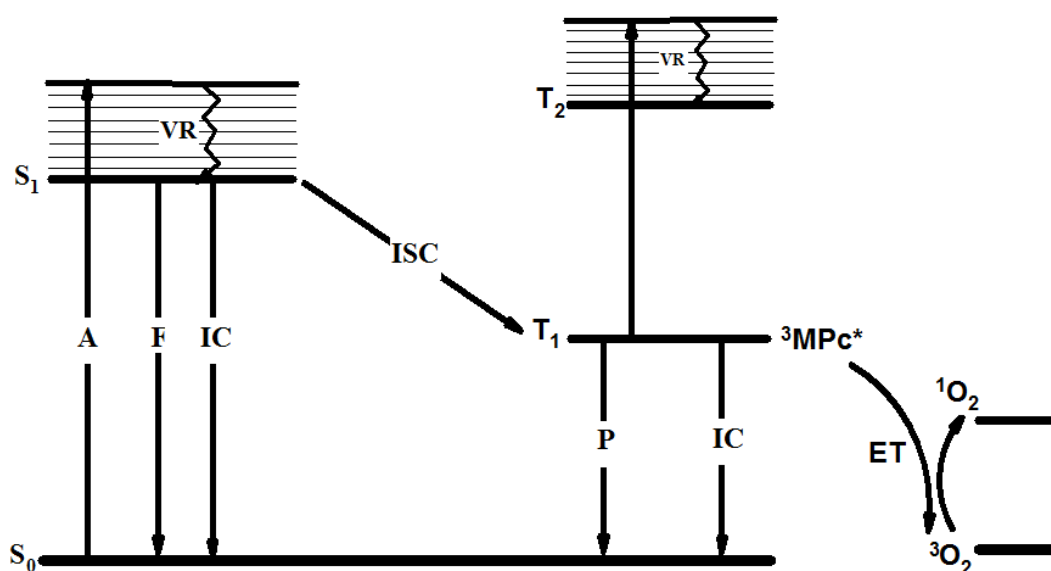


Figure 1.8: A modified Jablonski diagram showing the transitions between the singlet ground state (S_0) and electronic excited states (S_1 and T_1). A = absorption, F = fluorescence, VR = vibrational relaxation, IC = internal conversion, ISC = intersystem crossing, P = phosphorescence. S_1 = singlet excited state and T_1 = triplet excited state. ET = energy transfer.

1.2.6.1 Fluorescence quantum yields and lifetimes

Fluorescence is one of the processes that take place when a phthalocyanine molecule relaxes back to the ground state (**Figure 1.7**). The fluorescence efficiency of a chromophore is defined by the fluorescence quantum yield and fluorescence lifetime [81]. The fluorescence quantum yield gives an indication of the fraction of molecules losing energy by emission. With the help of a compound with a known fluorescence quantum yield (the standard), a comparative method is used to quantify the fraction of fluorescing molecules, **Equation 1.1** [82,83].

$$\phi_F = \phi_F^{std} \frac{F}{F_{std}} \frac{A_{std}}{A} \frac{n^2}{n_{std}^2} \quad (1.1)$$

where F and F_{std} are areas under the fluorescence emission curves of sample, and the standard, respectively. A and A_{std} are the absorbances of the samples and standard at the excitation wavelength; n and n_{std} are refractive indices of solvents used for samples and standard, respectively. In this work, ZnPc in DMSO was used as a standard, $\phi_F = 0.20$ [82]. The fluorescence lifetime (τ_F), determines the average time a molecule spends in the excited state before losing energy through emission. It is another important parameter that defines the efficiency of a fluorophore. A number of techniques are available for the determination of fluorescence lifetimes, but time correlated single photon counting (TCSPC) is the most popular and widely used technique. Details of the technique are discussed in the experimental section. The fluorescence lifetimes of

MPc complexes are short, with a typical time scale of the order of nanoseconds (ns, 10^{-9}) [82].

1.2.6.2 Triplet quantum yields and lifetimes

One of the most common fates for an optically excited organic molecule is the intersystem crossing (ISC) to populate the triplet state. Triplet quantum yield (ϕ_T) defines the probability of this process, therefore, it is a centrally important parameter in molecular photophysics. Triplet quantum yields can be defined as the number of molecules that undergo intersystem crossing from the first excited singlet state to the triplet state. Phthalocyanine triplet quantum yields and lifetimes are generally determined by laser flash photolysis [84]. The idea behind flash photolysis is to rapidly introduce an intense pulse of light to an absorbing species to produce sufficient concentration of the excited molecule in the triplet state, and then analyse the time evolution of the system by absorption spectroscopy [84]. The signal originates from the electronic transition from T_1 to T_2 (**Figure 1.7**). The information on the triplet quantum yield (ϕ_T) and triplet lifetimes (τ_T) may be obtained. The triplet quantum yield of a phthalocyanine can be obtained by a comparative method, **Equation 1.2**, with unsubstituted ZnPc as a standard [85].

$$\phi_T^{sample} = \phi_T^{std} \frac{\Delta A_T^{sample}}{\Delta A_T^{std}} \frac{\varepsilon_T^{std}}{\varepsilon_T^{sample}} \quad (1.2)$$

where A_T^{Sample} and A_T^{Std} are the changes in the triplet state absorbance of the sample and the standard, respectively. $\epsilon_T^{\text{Sample}}$ and ϵ_T^{Std} are the triplet state extinction coefficients for the sample and standard, respectively. ϕ_T^{std} is the triplet state quantum yield for the standard. The triplet quantum yield of the ZnPc standard in DMSO is = 0.65 [85]. A triplet decay curve can be fitted with Origin program to obtain the triplet lifetimes.

1.2.6.3 Singlet oxygen quantum yields

Singlet oxygen ($^1\text{O}_2$) is a higher energy state of molecular oxygen. It is one of the most reactive intermediates involved in chemical and biochemical reactions. The main method of producing $^1\text{O}_2$ is by photosensitization reactions. On populating the triplet state, the phthalocyanine can lose energy by phosphorescence or by energy transfer to the ground state molecular oxygen ($^3\text{O}_2$), converting it to the highly reactive $^1\text{O}_2$ (Figure 1.7). Experimentally, the singlet oxygen quantum yield (ϕ_Δ) of phthalocyanines is determined using optical or chemical methods [86]. The optical method involves the observation of a time-resolved phosphorescence decay of singlet oxygen at 1270 nm [87]. The chemical method is the most commonly used for quantifying singlet oxygen, and it requires the use of singlet oxygen quenchers that react rapidly with the singlet oxygen generated by the sensitizer in a 1:1 ratio without the possibility of side reactions taking place. The decomposition products of the quencher should neither react nor interfere with the detection of singlet oxygen [86]. In this work, 1,3 diphenylisobenzofuran (DPBF) or anthracene-9,10-diyl-

bismethylmalonate (ADMA) singlet oxygen quenchers were employed. Singlet oxygen quantum yields can then be determined using the comparative method (Equation 1.3).

$$\phi_{\Delta} = \phi_{\Delta}^{std} \frac{R}{R^{std}} \frac{I_{abs}^{std}}{I_{abs}} \quad (1.3)$$

where ϕ_{Δ}^{std} is the singlet oxygen quantum yields for the standard ZnPc (ϕ_{Δ} = 0.67 in DMSO [88]), R and R^{std} are the quencher's photobleaching rates in the presence of the sample and standard, respectively. I_{abs} and I_{abs}^{std} are the rates of light absorption by the sample and standard, respectively. Alternatively, the absolute method can be used to quantify singlet oxygen. This method is used when no suitable standard is available. The decrease in the absorption bands of the singlet oxygen quencher is spectroscopically monitored, and the quantum yield ($\phi_{quencher}$) is calculated using Equation 1.4 [89].

$$\phi_{quencher} = \frac{(C_o - C_t)V_t}{I_{abs}t} \quad (1.4)$$

where C_o and C_t are concentrations of the quencher (ADMA or DPBF) before and after irradiation respectively; V_t is the volume of the solution irradiated and I_{abs} is defined by Equation 1.5.

$$I_{abs} = \frac{\alpha A I}{N_A} \quad (1.5)$$

where $\alpha = 1 - 10^{-A(\lambda)}$, $A(\lambda)$ is the absorbance of the sensitizer at the irradiation wavelength, A is the irradiation area (2.5 cm^2), I is the intensity of light and N_A is the Avogadro's constant. The singlet oxygen quantum yield (ϕ_Δ) is calculated using Equation 1.6 [89].

$$\frac{1}{\phi_{\text{quencher}}} = \frac{1}{\phi_\Delta} + \frac{1}{\phi_\Delta} \frac{k_d}{k_a} \frac{1}{[\text{quencher}]} \quad (1.6)$$

where k_d is the decay constant of singlet oxygen and k_a is the rate constant of the reaction of the quencher with $\text{O}_2 (^1\Delta_g)$. The intercept obtained from the plot of $1/\phi_{\text{quencher}}$ versus $1/[\text{quencher}]$ gives the ϕ_Δ value.

1.2.7 Photophysical properties of phthalocyanines in the presence nanoparticles.

As mentioned previously, the core structure of the phthalocyanine ring can be modified with different substituents to improve the photophysical properties of the Pc. Insertion of different heavy metals into a Pc cavity can also improve the photophysical properties. In addition to these strategies, phthalocyanines have been used as capping ligands for various nanoparticles. **Table 1.1** lists a few phthalocyanines that have been conjugated to different nanoparticles [41,90-107]. When Wieder *et al.* conjugated gold to C11Pc (see **Table 1.1** for the full name), a 20% increase in the singlet oxygen quantum yield was observed [92]. Similar findings were reported by Modisha *et al.* when magnetic Fe_3O_4 nanoparticles were conjugated to $\text{ZnPc}(\text{COOH})_8$, where the increase in the singlet oxygen was accompanied by the

reduction in the fluorescence quantum yield [100]. This is very common since the two processes, fluorescence and intersystem crossing, are complimentary. The heavy atom effect induced by the presence of nanoparticles is largely responsible for the improved photochemical behaviour of the phthalocyanine. In this work, silver and ZnO nanoparticles were synthesized and conjugated to phthalocyanines. These two types of nanoparticles were chosen because they have been reported to have therapeutic and photocatalytic effects as discussed above [16-18,30-33]. Table 1.1 shows very little data is available on the photophysical and photochemical behaviour of phthalocyanines when conjugated to silver or ZnO nanoparticles. While there have been reports where ZnO or AgNPs have been conjugated to phthalocyanines [40,41,94,106,107], Table 1.1 shows that the application of these conjugates for PDT or photocatalysis is limited. Available reports on phthalocyanine-ZnO conjugates focus mainly on the photovoltaic properties of these hybrid materials [41], while few reports are available on photocatalysis, photophysical and photochemical behaviour of the conjugate is not reported.

Table 1.1: *Phthalocyanines conjugated to various nanoparticles*

MPc	NPs	Photophysicochemical parameters				Application	Ref.
		ϕ_F		ϕ_Δ			
		Pc	Pc-NPs	Pc	Pc-NPs		
Pc4	Au	–	–	–	–	<i>in vitro</i> PDT	90
HDMPZnPc	Au	–	–	–	–	PDT	91
C11ZnPc	Au			0.45	0.65	<i>in vivo</i> PDT	92,93
HDTSiPc	Ag	0.34	0.21	0.44	0.55	Bacterial inactivation	94
THdZnPc	Au	–	–	0.59	0.74	–	95
CuTSPc	TiO ₂	–	–	–	–	Photocatalysis	96
CoTSPc	TiO ₂	–	–	–	–	Photocatalysis	97
ZnPc	TiO ₂	–	–	–	–	Photocatalysis	98
FePc	TiO ₂	–	–	–	–	Photocatalysis	99
ZnPc(COOH) ₈	Fe ₃ O ₄	0.15	0.09	0.24	0.31	–	100
ZnPc(COOH) ₄	UCNPs	–	–	–	–	<i>In vivo</i> PDT	101
Pc4	Si	–	–	–	–	<i>In vitro</i> PDT	102
TCPZnPc	Si	0.18	0.11	0.50	0.50	–	103
AlPc	Si	–	–	–	–	–	104

ZnPc	Si	-	-	-	-	In vitro PDT	105
ZnPc	ZnO	-	-	-	-	Photocatalyst	40
CoPc	ZnO	-	-	-	-	Photocatalyst	106,107
ZnPc	ZnO	-	-	-	-	Solar cells	41
CuPc							

HDMPZnPc=tetrakis-(1,6-hexanedithiol)phthalocyaninato]zinc(II) ,C11ZnPc = tetradecakisdecyl dithiodiundecyl) diphthalocyanine zinc, HDTSiPc = bis-(1,6-hexanedithiol) phthalocyanine, THdZnPc=1,6-hexanedithiol zinc phthalocyanine ,ZnPc(COOH)₈ = zinc octacaboxy phthalocyanine, ZnPc(COOH)₄ = zinc tetracaboxy phthalocyanine, Pc-4= silicone phthalocyanine. CoTSPc = cobalt tetrasulfonated phthalocyanine, CuTSPc = copper tetrasulfonated phthalocyanine, AlPc = aluminium phthalocyanine, ZnPc= Zinc phthalocyanine, CoPc= cobalt phthalocyanine, ZnTSPc = zinc tetrasulfonated phthalocyanine

1.3 Phthalocyanines in photodynamic therapy of cancer

Although light has been used for therapeutic purposes for over centuries, the cornerstone of photodynamic therapy (PDT) was set in 1900 by Oscar Raab when he reported that acridine orange destroys microorganisms under irradiation with light [108]. A decade later, Friedrich Meyer-Betz injected himself with a porphyrin to test whether it made humans sensitive to light [109]. Today, PDT is used in the treatment of different ailments which include cancer. The main features that a photosensitizer (PS) must meet to have high photodynamic therapeutic efficacies are: low toxicity in the dark and high phototoxicity when activated with light, high selectivity and specificity for the target tumor, rapid elimination from the body, absorption in the optical transmission window for biological tissues, high quantum yield for singlet oxygen production, solubility in a water-based media and must be stable under physiological conditions [110]. Porphyrins form the first generation photosensitizers

(PS), where Photofrin[®] (**Figure 1.9**), is a well-known first generation PS approved for the treatment of lung, bladder, gastric and cervical cancers [111]. Although porphyrin based photosensitizers have shown remarkable photodynamic activity, clinically available first generation PS such as Photofrin[®] have some deficiencies such as weak absorption in the therapeutic window [111]. Second generation photosensitizers, such as chlorins and phthalocyanines (**Figure 1.9**), have a strong absorption in the red spectral region where tissue is more transparent [111]; therefore addressing the major limitation of the first generation PS. An example of a chlorin that has shown promise in clinical trials is Foscan (**Figure 1.9**). Foscan has been used in the treatment of head and neck squamous cell carcinoma [111]. In this work, we focused mainly on phthalocyanines for PDT. Phthalocyanines are popular second generation PS that have been approved in some countries, such as Russia, for the treatment of cancer. **Figure 1.9** shows some phthalocyanines that are in clinical trials [112]. **Table 1.1** shows that little work has been reported on the photodynamic therapy of phthalocyanines in the presence of nanoparticles, with the majority of reports focusing on gold nanoparticles.

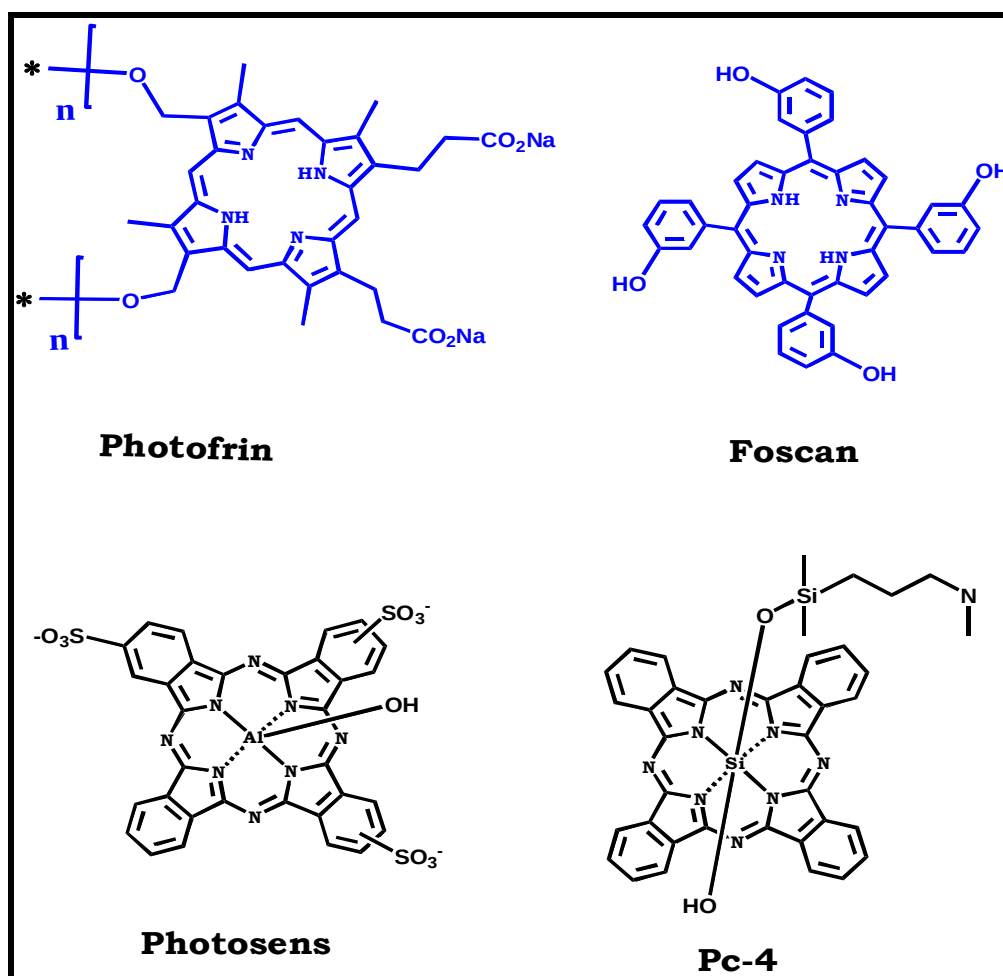


Figure 1.9: Some selected first and second generation photosensitizer in clinical trials.

Similar to first generation PS, second generation photosensitizers also suffer from the lack of selectivity. Although both 1st generation and 2nd generation photosensitizers accumulate with some selectivity into tumors, they also concentrate largely in normal tissues. Improved selectivity of any therapeutic implies that the dose of the drug reaching the diseased tissue is increased, while the dose reaching the normal tissues is decreased. This has then led to the development of tumor specific photosensitizers, also referred to as third generation photosensitizers [113]. A tumor specific therapeutic can be developed in a number of ways including the attachment of a cancer specific molecule onto a therapeutic drug. A tumor specific/ targeting

molecule can be a peptide, hormone, vitamin, monoclonal antibody or nanoparticles [113,114]. In this work the targeting efficiency of silver nanoparticles, folic acid and polylysine are investigated.

1.3.1. Folic acid

Tumors result from the uncontrolled proliferation of cells. These rapidly growing cells require metabolic substrates for nourishment and removal of metabolic end products. Folic acid (pteroyl-L-glutamic acid, **Figure 1.10**) or vitamin B9 is essential for cell replication and cell growth. Folic acid is acquired by cells through the presence of folate receptors (FRs) [113]. Since tumor cells grow quickly, their vasculature tends to differ greatly from normal cells. During the rapid cell proliferation, the majority of malignant cells overexpress folate receptors and other antigens that help them multiply more quickly [113]. Thus, cancer cells that are most difficult to treat with classical methods can be easily targeted with folate linked therapeutics [115,116]. Folate receptors are over expressed in a number of carcinomas, including malignancies of the ovary, brain, kidney, breast, colon and lung [117,118]. A wide range of folate drug conjugates have been reported to date, including folic acid linked to low molecular weight chemotherapy drugs and nanoparticles [117-120]. **Figure 1.10** shows the structure of folic acid and the moiety that is recognized by FR which is pterine (**Figure 1.10**). Benzoglutamic acid residue is used for anchoring different therapeutics onto folic acid [121].

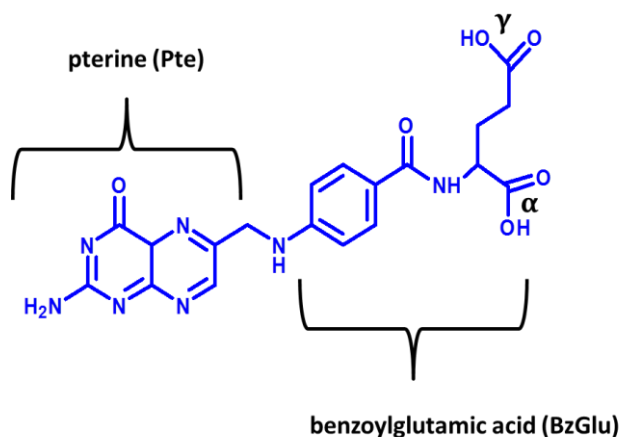


Figure 1.10: Chemical structure of folic acid.

The exploitation of folate receptor (FRs) mediated drug delivery has been referred to as a molecular Trojan horse whereby a therapeutic drug attached to folate is shuttled into a FR-positive cell. The drug-folic acid conjugate is internalized into the cell through receptor-mediated internalization (**Figure 1.11**). In this process, the targeting molecule attaches to the receptors (1), a signal is sent to the cell causing the internalization of the receptors forming early endosomes (2). This is followed by the dissociation of the conjugate and receptors (3) which is facilitated by the drastic changes in pH, and the receptors are recycled back to their place on the surface of the cell (4) [118,120].

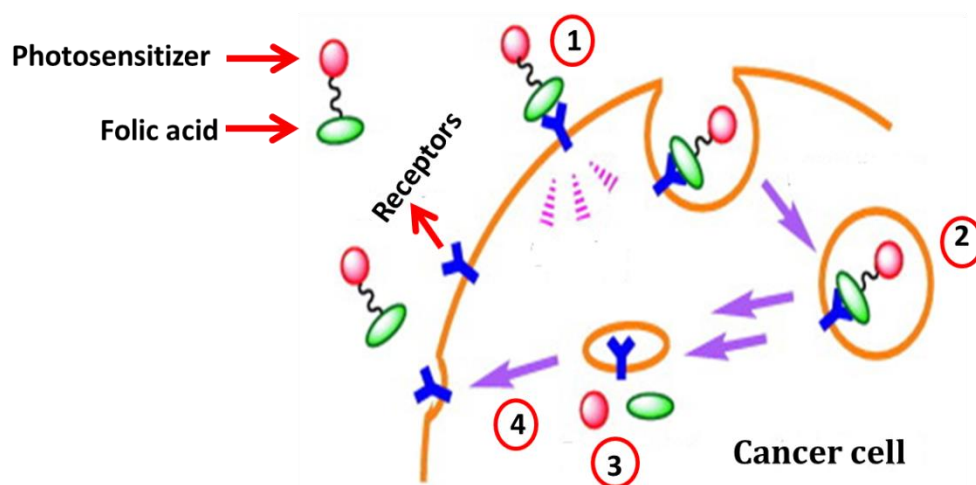


Figure 1.11: Internalization of folic acid-drug conjugate by endocytosis [122].

In this study, the properties of the Pcs are investigated upon conjugation to folic acid (a folate). Other than the work done by Qualls and Thompson [122], Nombona and Nyokong [123], **Table 1.2**, very little effort has been devoted to synthesizing phthalocyanines conjugated to folic acid. In the reported work, Pcs were mixed with folic acid; we therefore, study the effect of chemically linking folic acid to phthalocyanines for the first time. The efficacy of the conjugate is investigated on breast cancer cell lines.

1.3.2 Polymer conjugates

Selective targeting is divided into active and passive targeting. Active targeting exploits the overexpression of some receptors, i.e. folate receptors, while passive targeting entails the exploitation of the imperfections of a tumor cell [124-126]. When

tumor cells multiply quickly, angiogenesis is induced to cater for the high demand of nutrients and oxygen [124,127]. The neovasculature of a tumor cell differs greatly from that of normal tissues. In tumor cells, the blood vessels are irregular in shape, dilated and leaky; the endothelial cells are poorly aligned with large fenestrations [125-128]. These anatomical defects result in leakage of the drugs (composing of macromolecules and nanoparticles) into tumor tissue. The poor lymphatic clearance means that macromolecules are retained in the tumor while extravasation continues. This forms the basis of enhanced permeability and retention (EPR) effect [124-129].

Polymers form an important class of directing agents that rely strongly on the EPR effect. Not only are polymers easily accumulated by neoplastic tissues, biocompatible polymers can infer biocompatibility onto a therapeutic drug. Several polymers such as poly(ethylene glycol) (PEG), N-(2-hydroxypropyl)methacrylamide (HPMA), poly(lactide-co-glycolide) (PLGA) have been successfully applied in selective tumor targeting [130-132]. In this work, poly-lysine is conjugated to a phthalocyanine with the aim of inducing water solubility, and increasing the selectivity of the phthalocyanine. Polylysine is commercially available, and can be easily modified for the attachment of therapeutic drugs. As shown in **Table1.2**, there are no reports on the photodynamic activity of phthalocyanines when conjugated to polylysine, particularly where cancer cells are employed. [133-135].

Table 1.2: *Phthalocyanines conjugated to folic acid and polylysine.*

MPc	Folic acid/ polylysine	Application	Reference
ALTSPc	Folic acid	<i>In vitro</i> PDT	122
MPyCaZnPc	Folic acid	—	123
MCaZnPc	Polylysine	Bacterial inactivation	133
ZnTSPc	Polylysine	Bacterial inactivation	134
CoTSPc	Polylysine	O ₂ sensor	135

ALTSPc= aluminium tetrasulfonated phthalocyanine, *MPyCaZnPc*= 9,16,23-tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyaninato zinc(II), *MCaZnPc*= monocarbocy zinc phthalocyanine, *TSZnPc* = tetrasulphonated zinc phthalocyanine, *ALTSPc*= zinc tetrasulfonated phthalocyanine, *CoTSPc* = cobalt tetrasulfonated phthalocyanine.

1.3.3 Cancer cells employed in this work

Cancer is a global term for a variety of diseases that are characterized by uncontrolled cellular growth, enhanced angiogenesis and reduced programmed cell death [136]. The site of origin of these diseased cells is used to define general categories of cancer (e.g. breast cancer, skin cancer, lung cancer, brain, etc.) [137,138]. While current cancer treatment methods are efficient, they suffer from a number of drawbacks, i.e. during chemotherapy both healthy and cancer cells are destroyed resulting in a number of undesirable side effects. PDT is a non-invasive alternative method. Therefore, research is now focused on optimizing the selectivity of the photosensitizers used in PDT.

In this study, three types of tumor cells (breast, prostate and melanoma) were used to assess the efficacy of the prepared antitumor drugs. Overall, the cell lines were selected based on their anatomy, i.e breast cancer cells overexpress folate receptors

[139,140], therefore, the efficacy of folic acid conjugates was investigated on MCF-7 breast cancer cells. Among other enzymes, cancer cells uniquely exhibit serine proteases which is capable of degrading polylysine backbone upon internalization [141-143]. This enzyme is reported to exist in many human tumors including lung, brain, head and prostate cancer, therefore, the efficacy of polylysine conjugates was investigated on prostate cancer cells [141-143]. Melanoma cancer cells were also used to study the potency of the photosensitizers. Initially, the strategy was to compare the potency of the photosensitizer on both normal (fibroblast) and cancerous (melanoma) skin cells. However, due to time constraints, the potency of the photosensitizer was only investigated on melanoma cancer cells.

1.4 Phthalocyanines as photocatalysts

Advanced oxidation process (AOP) is receiving a great deal of popularity owing to its efficiency in the degradation of recalcitrant dye pollutants in wastewater [144-146]. The method is based on the use of reactive oxygen species (ROS) in mineralizing pollutants in water [143,147]. Photocatalysis is one of the eminent methods in the degradation of organic dyes. The efficiency of the method is mainly dependent on the type of catalyst used [148-150]. There have been reports on the use of phthalocyanines as photocatalysts in the photodegradation of organic dyes [151,152]. The photocatalytic application of phthalocyanines arises from their ability to produce ROS upon irradiation with the appropriate wavelength of light [152,153]. In this study, we focus on the ability of Pcs as photocatalysts when conjugated to nanoparticles and

immobilized onto different supports. Phthalocyanines were immobilized onto electrospun fibers and chitosan beads for ease of recovery and recyclability. The chosen supports offer a number of advantages that will be discussed later. As can be seen in **Table 1.3**, our group and others have reported on phthalocyanines supported onto electrospun fibers, but little information is available on the photocatalytic behaviour of phthalocyanine-nanoparticle conjugates immobilized onto chitosan beads or fibers [151-163].

1.4.1 Electrospun fibers preparation

The idea of immobilizing phthalocyanines onto fibers was well described by Amanbaeva [164]. Amanbaeva demonstrated that if the phthalocyanine containing polymer is made porous, it can be used as a catalyst in the treatment of waste water. The porosity of the polymer can be easily achieved by electrospinning the polymer into fibers as is the case in this work. Electrospun polymer fibers are characterized by large surface area to volume ratios, and the high porosity allows for effective interaction between the embedded phthalocyanine molecules and photons of light [164,165]. Different methods such as: drawing, template synthesis, self-assembly and phase separation, are available for the fabrication of polymeric fibers [166-169]. However, electrospinning is a widely used technique for the fabrication of continuous fibres [166,170-172]. The ability to fabricate fibers with possible control of the fiber fineness, surface morphology and cross sectional configuration give electrospinning an

edge over other processes. In addition, electrospinning is simple, convenient and the fibers prepared by this method can be reproduced [172].

In the electrospinning process, fibers are generated through an electrically charged jet of polymer solution. The principles governing electrospinning are similar to electrospraying. A simple electrospinning set-up is shown in **Figure 1.12**. Experimentally, the apparatus for electrospinning consists of three basic components: (1) A syringe for the polymer solution, (2) a high voltage power supply, and (3) a conductive collector plate where the fiber mat is deposited [165,169-172]. The process begins with the polymer solution being pumped out of the syringe through the needle to form a drop at the tip of the needle. The drop is charged by applying a high voltage on the needle, the applied voltage induces Coulombic forces which will counteract the surface tension that holds the drop in the spherical shape, thus, distorting it into the so-called 'Taylor cone' [170-173]. When the electric field exceeds a certain value, a charged fluid jet is ejected from the tip of the Taylor cone in the form of fibers, where they are collected as unwoven mat [172,173].

Phthalocyanines are immobilized onto fiber mats by either a physical or chemical method. Systems in which a phthalocyanine is primarily bound to the support by sorption forces constitute the group of physically supported phthalocyanines. Phthalocyanines can also be incorporated inside a polymer matrix during the synthesis of the polymer [153]. It is highly desirable to explore environmental benign and simple methods for the immobilization of phthalocyanines to produce catalysts

which are truly efficient and readily recycled. Therefore, in this study, phthalocyanines were supported into the fiber mat by using a simple physical method.

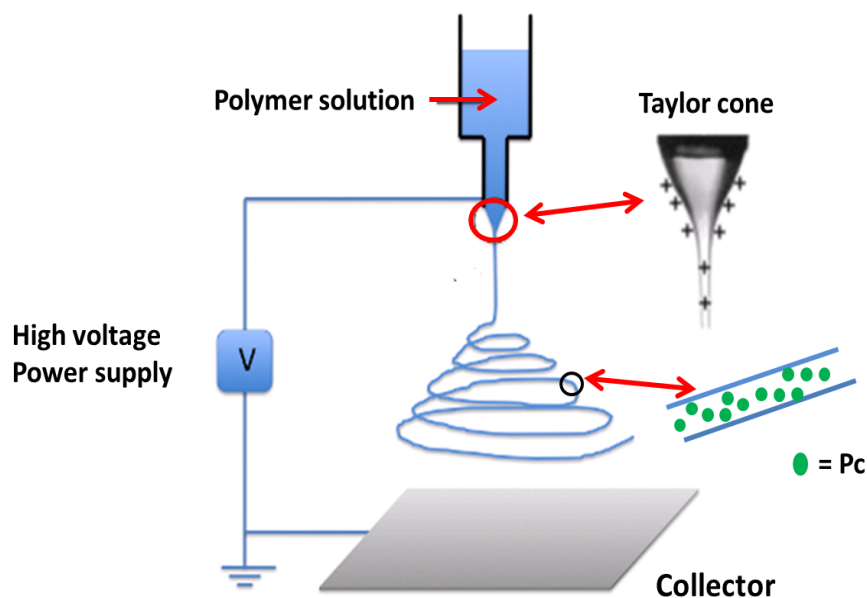


Figure 1.12: Typical diagrammatic set-up of electrospinning apparatus.

1.4.2 Chitosan microbeads

Chitosan microbeads were also employed as supports in this study. Microbeads (in general) were initially developed for drug delivery; therefore, they are usually made from natural or synthetic polymers that are biodegradable in nature [174-176]. Polymers such as gelatin, collagen, chitosan and others have been used for the preparation of microbeads [177-179]. Chitosan, a derivative of chitin, is one of the biodegradable polymers that have been used in drug delivery. Furthermore, due to its abundance, biodegradability and non-toxicity, chitosan has landed applications in different fields [177-179]. It exhibits a strong affinity for transition metals; therefore, in addition to biological applications, it has been widely used in water purification [180-

183]. A number of researchers have functionalized chitosan for the preparation of heterogeneous catalysts in the form of colloids, fibers and microbeads [**183-186**]. However, only a few reports are available on the immobilization of phthalocyanines onto chitosan beads for the removal of organic pollutants, **Table 1.3**, [**162,163**].

Several strategies can be followed to prepare chitosan gel beads. Methods such as ionotropic gelation, wet phase inversion, emulsification, ionotropic gelation and coacervation are reported in literature [**183,187**]. The coacervation precipitation method is the simplest and most convenient method for the preparation of beads, and it was chosen for the preparation of the beads used in this study. In this process, the polymer is solubilized, followed by the drop-wise addition of this solution into an alkaline media that precipitates it out [**187**]. The method of coacervation is based on the physicochemical property of chitosan, since it is insoluble in alkaline media, therefore, it precipitates out when in-contact with an alkaline solution [**187**].

In this work, a silver nanoparticle-phthalocyanine conjugate was immobilized onto chitosan beads, for the first time. The chitosan gel beads serve as porous scaffolds accessible by substrates (dye pollutant) and porous to the products of the reaction, but they are sufficiently packed to retain the photocatalyst [**183-187**]. The question being addressed by the study is: are the photochemical properties of phthalocyanine-silver nanoparticle conjugate retained when confined in the microbeads?

Table 1.3: *Phthalocyanines-nanoparticle conjugates supported on fibers or chitosan beads for dye removal.*

MPc	NPs	Type of support	Dye	Ref.
LuTPPc	-	polystyrene fibers	4-chlorophenol	151
LuTPyPc		polystyrene fibers	4-nitrophenol	158
ZnTBMPc	Au	polystyrene fibers	4-chlorophenol	152
ZnTPyPc	-	Polystyrene fibers	4-chlorophenol	153
LuTPyPc		Polysulfone fibers	4-nitrophenol	
ZnTAPc		Polyurethane fiber	Methyl orange	
LuTAPc		Polyacrylic acid fibers		
Zn(COOH) ₈	Fe ₃ O ₄	polystyrene fibers	Orange G	159
FeTNPc	TiO ₂	Polyvinylpyrrolidone	Methyl orange	160
CoTAPc		Cellulose fiber	Reactive Red X-3B	161
FeTSPc	-	Chitosan beads		162
FeTSPc	-	Chitosan beads	β-isophorone	163
CoTSPc				

LuTPPc = tetraphenoxy phthalocyanine, LuTPyPc= tetrapyridyloxy phthalocyanine, ZnTBMPc = tetra-benzylmercapto phthalocyanine, Zn(COOH)₈=tetracarboxy phthalocyanine, ZnTAPc= tetraamino phthalocyanine FeTNPc = tetranitro phthalocyanine, CoTSPc= tetrasulphonated phthalocyanine.

1.4.3 Brief description of dye pollutants employed in this work

Dye pollutants are a serious source of environmental contamination. The efficiency of the prepared immobilized catalysts was examined for the degradation of Rhodamine 6G (Rh 6G) and methyl orange, which represent two different classes of dyes. The structures of these model dyes are depicted in **Figure 1.13**.

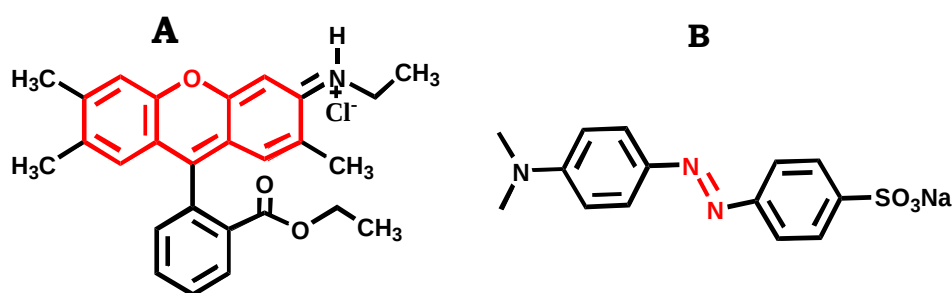


Figure 1.13: Chemical structure of Rhodamine 6G (A) and methyl orange (B).

Rhodamine 6G is a cationic and lipophilic fluorescent dye and it belongs to a class of dyes called Xanthene [188]. These dyes are often used as tracers when determining the rate and direction of the water flow. They also serve as biological stains, solar collectors, laser, and paper dyes [189]. Xanthene dyes are harmful to both humans and animals. They cause irritation of the skin, eyes and respiratory tract. Their carcinogenicity, reproductive and chronic toxicity have been experimentally proven [189,190]. Because they are fairly stable, they have become common organic pollutants. Therefore, it is crucial to find a photocatalytic system that is able to decompose Rh 6G to smaller, less toxic, and biodegradable species.

Waste waters from the textile industries contain many organic and inorganic substances and are one of the main sources of environmental pollution due to the rapid development of industries. Dyes such as anthraquinone, arylmethane, indigoid and azo dyes constitute a class of dyes that are used in large scale in the textile industries [191]. In this study, the efficiency of the prepared catalysts was investigated on methyl orange, a dye pollutant that falls under recalcitrant azo dyes. In addition to textile industry, azo dyes are also used in cosmetics, ink and in paint industry [191]. These dyes are characterized by the presence of an azo group ($-N=N-$) bound to aromatic rings [191-193]. The azo group gives them intense colouring, therefore, their release to natural waters is of great concern. Azo dyes are also reported to be toxic, carcinogenic and mutagenic [192,194].

Up until now, different methods have been developed for removing coloured pollutants. Methods commonly employed include coagulation/flocculation, air stripping, incineration, reverse osmosis, ultrafiltration and ion exchange on synthetic adsorbent resins [195]. A disadvantage of these processes is that they are non-destructive; they only transfer the pollutant from one phase to another, thus, causing secondary pollution. In this study, the phthalocyanines' ability to degrade organic pollutants was investigated, mainly because they fall under an important class of catalysts, i.e. APOs that remove dye pollutants by degradation to less harmful by-products.

1.5 Summary of Aims

1. Synthesize and characterize novel phthalocyanines, with substituents that exhibit anticancer properties such as carbazoles, benzothiazole and ketal ligands.
2. Carry out detailed spectroscopic studies (ground state electronic absorption, fluorescence emission and excitation spectra) of the new molecules.
3. Study the photophysical (fluorescence and triplet quantum yields and lifetimes), and photochemical (singlet oxygen) behaviour of these novel phthalocyanines.
4. Improve the selectivity of phthalocyanines by conjugation to targeting molecules such as folic acid and poly-L-lysine.
5. Characterize and investigate the photophysicochemical behaviour of phthalocyanine when conjugated to targeting molecules.
6. Prepare and characterize silver and ZnO nanoparticles.

7. Conjugate the novel phthalocyanines to Ag or ZnO nanoparticles (by adsorption or covalent bond formation) and investigate their spectroscopic behaviour.
8. Study the photophysical behaviour of the phthalocyanines when conjugated to nanoparticles.
9. Apply the phthalocyanines and its conjugates in photodynamic therapy of cancer cells.
10. Immobilize the phthalocyanines and phthalocyanine-nanoparticle conjugate onto different supports through physisorption.
11. Characterize the immobilized phthalocyanines and phthalocyanine-nanoparticle conjugates.
12. Study the photocatalytic behaviour of the immobilized catalysts using Rhodamine 6G and methyl orange as environmental contaminants.

CHAPTER 2

EXPERIMENTAL SECTION

2.1 Materials

2.1.1 Solvents

Chloroform, dichloromethane (DCM), diethyl ether, *N, N* dimethylformamide (DMF), 1-pentanol, acetone, dimethylsulfoxide (DMSO), ethanol (EtOH), n-hexane, methanol (MeOH), tetrahydrofuran (THF), acetonitrile (ACN), toluene, nitric acid (55 %), sulfuric acid (98 %), pyridine, and thionyl chloride were purchased from SAARCHEM. Ammonia (25 %), deuterated DMSO (DMSO-*d*₆), trifluoroacetic acid (TFA), deuterium oxide (D₂O) were purchased from Sigma-Aldrich. Ultra-pure water was obtained from Milli-Q water system (Millipore Corp., Bedford, MA, USA).

2.1.2 General reagents

1,3-Diphenylisobenzofuran (DPBF), 1,8-diazabicyclo{5.4.0}-undec-7-ene (DBU), zinc phthalocyanine (ZnPc), dicyclohexylcarbodiimide (DCC), anthracene-9,10-diyl-bismethylmalonate (ADMA), potassium carbonate, silica gel 60P F₂₅₄ (for column chromatography), sodium citrate (>99.0 %), zinc acetate, NaOH, 2-hydroxybenzothiazole, 2-hydroxycarbazole, iodomethane, sodium dodecyl sulfate (SDS), poly-L-lysine (PLL) HBr (70, 000), folic acid and N-hydroxysuccinide (NHS) were purchased from Sigma-Aldrich. 3- nitrophthalonitrile (**1**) and 4-nitrophthalonitrile (**2**) were synthesized according to reported methods [58,59].

Complex **10** and **11**, **Figure 1.4**, were also prepared according to methods reported in literature [196, 197].

2.1.3 Reagents for cell culture

Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, heat-inactivated fetal calf serum (FCS), penicillin-streptomycin with 1 µg/mL amphotericin B (PSA) and Dulbecco's phosphate-buffered saline (DPBS) were obtained from Whitehead Scientific (Pty) Ltd, South Africa. Cell proliferation reagent, WST-1, was acquired from Roche, South Africa. PC-3M-luc-C6 (Pc-3) cell lines of prostate cancer were a generous gift from Caliper Life Sciences (Alameda, CA, USA). Melanoma cancer cell lines (A375) and trypan blue were purchased at Sigma Aldrich, South Africa, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Sigma Aldrich, Buchs, Switzerland.

2.2 Instrumentation

- i. **Ground state electronic absorption spectra** were recorded on a Shimadzu UV-2550 UV/Vis spectrophotometer. Quartz cell with 1 cm path-length was employed. Absorbance of the phthalocyanines immobilized onto electrospun was measured by dissolving the fibers in toluene.

- ii. **Elemental analyses** were acquired on a Vario-Elementar Microcube ELIII Series.
- iii. **Infrared (IR)** spectra were recorded on a Perkin-Elmer (ATR) Fourier transform-IR (2000 FT-IR) spectrophotometer.
- iv. **^1H -nuclear magnetic resonance spectra** (^1H -NMR) were recorded in deuterated solvents ($\text{DMSO-}d_6$ or D_2O) using either a Bruker AMX400 MHz NMR spectrometer or a Bruker AVANCE II 600 MHz spectrometer.
- v. **Mass spectral** data was collected with a Bruker AutoFLEX III Smart beam TOF/TOF mass spectrometer. The instrument was operated in positive mode. 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 α -cyano-4-hydroxycinnamic acid as the MALDI matrix, and a 355 nm Nd: YAG laser as ionizing source.
- vi. **Scanning electron microscope (SEM)** images of electrospun polymer fibers and microbeads were obtained using a JOEL JSM 840 scanning electron microscope.

- vii. **Fluorescence micrographs** Fluorescence micrographs of phthalocyanine and phthalocyanine-nanoparticle functionalized fibers were taken by placing the fiber on a glass slide. The fluorescence images were taken with a Direct Metal Laser Sintering (DMLS) fluorescence microscope. The excitation source was a high voltage mercury lamp and light in the wavelength range of 550 – 730 nm. A digital camera was used to obtain micrographs.
- viii. **Transmission electron microscope (TEM)** images were obtained using a Carl Zeiss Libra transmission electron microscope operating at 100 kV accelerating voltage.
- ix. **X-ray powder diffraction (XRD)** patterns were recorded on a Bruker D8 Discover equipped with a Lynx Eye Detector, using Cu-K α radiation ($\lambda = 1.5405$ Å, nickel filter). Data was collected at various 2θ ranges (5-100°) 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 zero background silicon wafer slide. The X-ray diffraction (XRD) data were treated using Eva (evaluation curve fitting) software. Baseline correction was performed on each diffraction pattern by subtracting a spline fitted to the curved background.
- x. **Fluorescence emission and excitation spectra** were obtained on a Varian Eclipse spectrofluorimeter.

- xi. Fluorescence lifetimes** were measured using a time correlated single photon counting setup (TCSPC), Fluo Time 200, Picoquant GmbH, with a diode laser as excitation source (LDH-P-670 driven by PDL 800-B, 670 nm, 20 MHz repetition rate). Fluorescence was detected under the magic angle with a peltier cooled photomultiplier tube (PMT) (PMA-C 192-N-M, Picoquant GmbH) and integrated electronics (PicoHarp 300E, Picoquant GmbH). A monochromator with a spectral width of about 4 nm was used to select the required measured emission wavelength. The response function of the system, which was measured with a scattering Ludox solution (DuPont), had a full width at half-maximum (FWHM) of about 300 ns. The ratio of stop to start pulses was kept low (below 0.05) to ensure good statistics. All luminescence decay curves were measured at the maxima of the emission peak. The data were analysed with the program FluoFit (Picoquant GmbH). The support plane approach [198] was used to estimate the errors of the decay times.
- xii. Time-resolved emission spectra (TRES)** were plotted from TCSPC traces obtained at different wavelengths using the steady state emission spectra.
- xiii. Laser flash photolysis** experiments, **Figure 2.1**, were performed with light pulses produced by a Quanta-Ray Nd: YAG laser providing 400 mJ, 9 ns pulses of laser light at 10 Hz, pumping a Lambda-Physik FL3002 dye laser (Pyridin 1 dye in methanol). Single pulse energy ranged from 2 to 7 mJ. The analysing beam source was from a Thermo Oriel Xenon arc lamp, and photomultiplier

tube (a Kratos Lis Projekte MLIS-X3) was used as a 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.

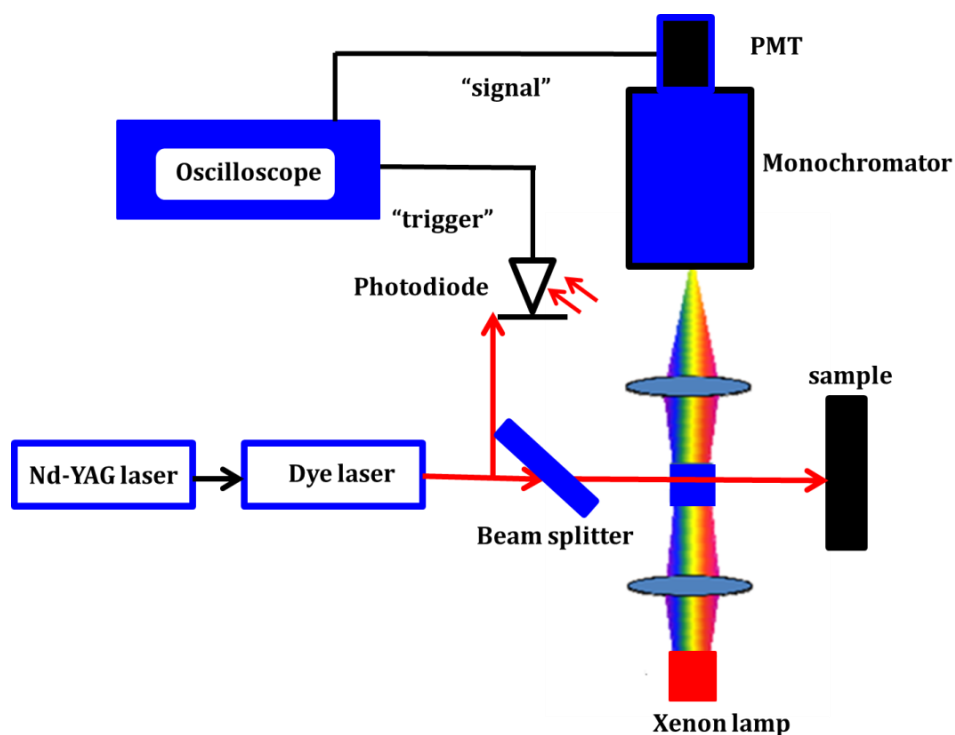


Figure 2.1: Schematic diagram for a laser flash photolysis setup. PMT = Photomultiplier tube.

- xiv. **Determination of singlet oxygen quantum yields** (chemical method) as well as phototransformation of organic pollutants was done using General Electric Quartz lamp (300 W) as irradiation source, **Figure 2.2**. A 600 nm glass (Schott) and water filters were used to filter off ultra-violet and far infrared radiations, respectively. An interference filter 670 nm with bandpass of 40 nm was placed in the light path just before the cell containing the sample. The intensity of light reaching the reaction vessel was measured with a power meter (POWER MAX 5100, Molelectron Detector Inc).

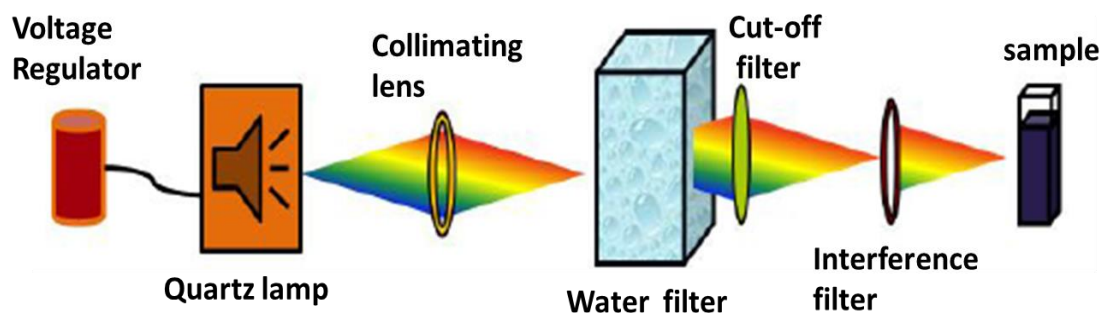


Figure 2.2: Schematic diagram of photo-irradiation setup.

- xv. **Electrospun polymer fibers** were obtained from a set-up, **Figure 2.3**, consisting of a high voltage source (Glassman High Voltage, Inc., EL series, 0-40 kV), a pump (Kd Scientific, KDS-100-CE), a glass syringe (Am Bildacker 3-7, D-97877 Wertheim, Poulten & Grat GmbH) connected to a steel needle with an internal diameter of 0.584 mm and aluminum foil as collector.

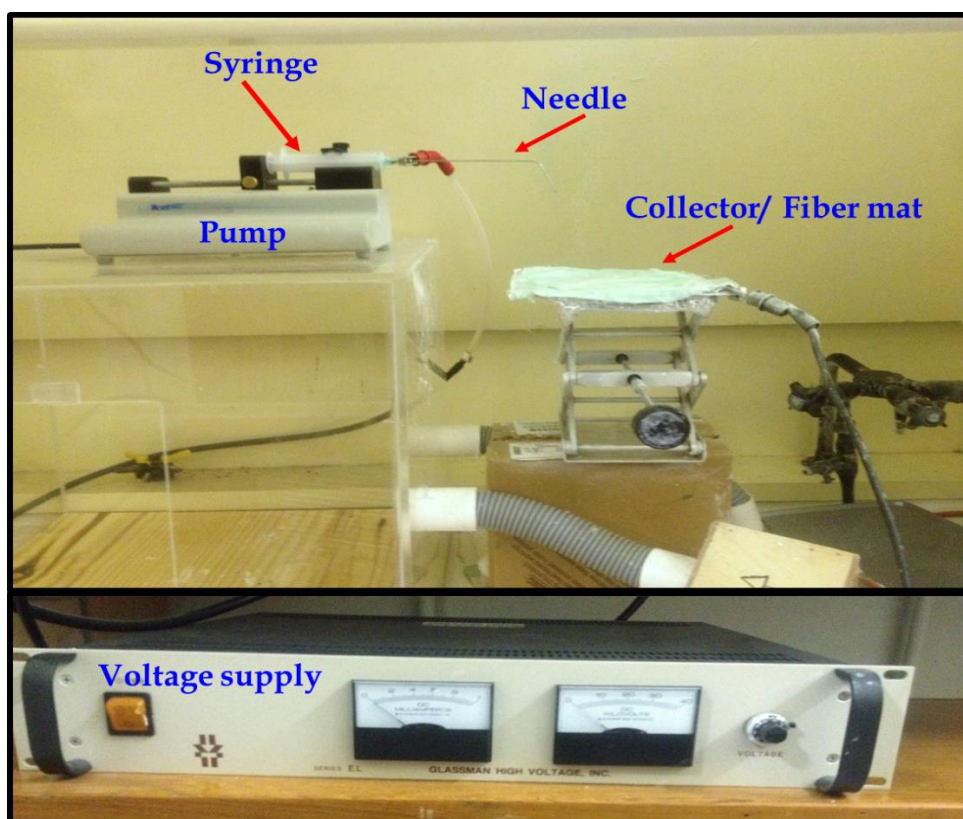


Figure 2.3: Typical electrospinning set-up.

- xvi. **High performance liquid chromatography (HPLC)** was used to profile the degradation products of Rhodamine 6G by comparison with the retention time of Rhodamine 6G at $t = 0$ min. Analysis of the products of Rhodamine 6G oxidation was confirmed with an Agilent HPLC 1200 series (Agilent Technologies Inc., Germany), equipped with a quaternary pump (G1311A), an autosampler (G1329A), a diode array detector (DAD SL G1315C) and a degasser unit (G1322A). An Eclipse XBD-C₁₈ (i.d. 4.6 x 150 mm; 5 μ m) column was used for the separation of the product intermediates. Gradient elution was used, starting with a mobile phase of a mixture of ACN-water (0.2 % acetic acid) (20/80 v/v) for 10 min which was then ramped to 100 % ACN at 20 min; the flow rate was kept at 0.8 mL/min and the injection volume was 25 μ L. The solvent was then kept at 100 % ACN for a further 10 min. The total run time was 30 min.
- xvii. **Morphology** of the cells was studied using inverted light microscopy, with an Axio Vert.A1 microscope. The absorbance of WST-1 was measured at 450 nm, using Synergy Mx monochromator-based multi-mode microplate reader. MTT absorption was measured at 525nm with a Sapphire, Tecan plate reader.

2.3 Methods

2.3.1 Fluorescence quantum yields (Φ_F)

Fluorescence quantum yields (Φ_F) of the MPcs and MPc conjugates were determined by a comparative method in DMSO using, **Equation 1.1**. The same solvent was used for the samples and standard. Unsubstituted ZnPc (std $\Phi_F = 0.20$ [82]) in DMSO) was employed as the standard. Both samples and standard were excited at the same wavelength. The absorbance of the solutions, at the excitation wavelength, was kept at about 0.05 to avoid any inner filter effects.

2.3.2 Triplet quantum yields (Φ_T) and lifetimes (τ_T)

The triplet decay kinetics of the triplet absorption of phthalocyanines were recorded using a laser flash photolysis setup (**Figure 2.1**). The absorbance of sample solutions and that of the standard were adjusted to be at approximately 1.5 at their Q-band maxima. After introducing the solution to a 1 cm quartz cell, argon was bubbled through the solution to remove dissolved oxygen before taking readings. The triplet quantum yields of the samples were determined using **Equation 1.2**. Unsubstituted ZnPc = 0.65 [85] in DMSO was employed as the standard. Triplet lifetimes were determined from the kinetic data obtained, using ORIGIN Pro 8 software.

2.3.3 Singlet oxygen (ϕ_{Δ}) quantum yields

A chemical method was employed in this work for ϕ_{Δ} determination, using the experimental set-up shown in **Figure 2.2**. The ϕ_{Δ} values were determined by monitoring a change in the absorbance of the singlet oxygen quencher, at 416 nm for DPBF and 380 nm for ADMA, with time. Values obtained were used to calculate ϕ_{Δ} according to **Equation 1.3** ($\text{ZnP } \phi_{\Delta} = 0.67$ in DMSO [88]) and **Equation 1.6**. Briefly, an air-saturated solution of the MPcs (2.0 mL) with the respective singlet oxygen quencher (DPBF or ADMA) was placed in a 1 cm path length spectrophotometric quartz cell, and photolysed at the Q-band region using a 300 W general electric quartz lamp as described above (**Figure 2.2**). The wavelength of the interference filter was chosen such that it was close to the Q band absorption of the MPc. The concentration of DPBF was kept at $\sim 3 \times 10^{-5}$ M to avoid chain reactions. The initial quencher concentrations (corresponding to an absorbance of ~ 1.0) were kept the same for both the standard and the MPcs.

For singlet oxygen quantum yield determinations carried out in water, e.g. where phthalocyanines were electrospun into fiber matrices, ADMA was used as a singlet oxygen quencher since the fabric materials are intended for use in aqueous media. In each case, the modified fibers (10 mg) or beads (0.2 g) were suspended in an aqueous solution of ADMA, and similarly irradiated using the photolysis set-up, **Figure 2.2**. The quantum yields were estimated using **Equation 1.6**. The values are estimates due

to light scattering, and the intensity of the light measured was that reaching the spectrophotometric cell but not the fibers.

2.3.4 Photocatalytic reactions

Photocatalytic reactions were carried out in a magnetically stirred batch reactor (glass vial). The irradiation experiments were carried out using the photolysis set-up described above for singlet oxygen detection, **Figure 2.2**. The intensity of light reaching the reaction vessel was measured to be 9.53×10^{18} photons $\text{s}^{-1} \text{cm}^{-2}$. The transformation was monitored (using a Shimadzu UV-2550 spectrophotometer) by observing the absorption bands of Rhodamine 6G at 525 nm and methyl orange at 470 nm after 10 and 30 min photolysis cycles, respectively. The experiments were carried out using a range of concentrations of Rhodamine 6G or methyl orange, each sample (6 mL) contained functionalized fibers (10 mg) or beads (0.2 g).

2.3.5 *In vitro* PDT cell studies

2.3.5.1 Cell culture

Prostate (Pc-3), melanoma (A375) and MCF-7 cancer cell lines were cultivated as monolayer in T75 cm^2 culture flasks in DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% amphotericin B and 1 % penicillin-streptomycin mixture. Cells were incubated at 37 °C in a 5% CO_2 humidified incubator.

2.3.5.2 Preparation of cells for experiments

After reaching 70-80 % confluence, cells were detached from the flask using trypsin. The activity of trypsin was quenched by adding fresh supplemented DMEM, and the cells were centrifuged for 2 minutes at 2000 rpm. Viable cells were counted with the neubauber hemocytometer cell counter, using trypan blue exclusion test of viability. A concentration of 10^4 cells was seeded and incubated with different concentrations of compounds for dark and phototoxicity studies. For experiments with complex **8** and **8**-AgNPs, concentrations of 10, 20, 40 and 80 μ M were used. For experiments with complex **10** and **10**-FA, concentrations 20, 40, 80, 160 and 360 μ M were employed; lastly, with complex **11** and **11**-polylysine, concentrations 1, 2, 4 and 8 μ M were studied. Cellular viability was conducted by incubating cells with neutral red, or with tetrazolium reagents WST-1 or MTT. Unless stated otherwise, data obtained represents the mean values from three independent experiments. Error bars are standard deviations.

2.3.5.3 Dark cytotoxicity

Cells were incubated for 24 hours with different concentrations of complex **8**, **8**-AgNPs, complex **10**, **10**-FA, complex **11** and **11**-polylysine. The media containing the photosensitizer was removed and cells were washed with DPBS. Fresh media was added and cells were further incubated for 24 hours. Assays outlined above were used

to quantify cellular viability; results are given in percentage survival relative to the untreated control (cells with no photosensitizer added).

2.3.5.4 Light toxicity

For the determination of laser cytotoxicity, cells were allowed to attach overnight (**without a photosensitizer**). Cells were then washed twice with DPBS and the culture media was replaced with the media without the phenol red. Irradiation was conducted using a diode laser with a wavelength of 676 nm, provided by the National laser centre (NLC), South Africa. The set-up described above was also used for illuminating the cells (**Figure 2.2**). After irradiation, cells were washed and incubated for an additional 24 hours. Fluencies for the irradiation ranged from 0.5 to 91.6 J/cm² [199]. The light beam was delivered from above and it covered the entire area of the petri dish. After irradiation, the media without phenol red was replaced with that containing phenol red and the cells incubated for 24 hours at 37 °C in 5% CO₂. Cell survival was expressed as the percentage of un-treated controls (0 J/cm²).

2.3.5.5 Photodynamic therapy (PDT) studies

Cells were incubated for 24 hours with different concentrations of complex **8**, **8**-AgNPs, complex **10**, **10**-FA, complex **11** and **11**-polylysine. Wells were washed twice with DPBS and the culture media was replaced with the fresh media (with no phenol red). The wells containing cells were illuminated, and after irradiation, the culture

media (without phenol red) was replaced with the fresh media containing phenol red. Cells were incubated for 24 hours at 37 °C in 5% CO₂, cellular viability was expressed as the percentage of un-treated controls.

2.4 Synthesis

2.4.1 Phthalonitrile synthesis

Scheme 3.1 outlines the synthetic procedures employed for the synthesis of phthalonitriles.

2.4.1.1 3-(2-Benzothiazol-2-yloxy)-phthalonitrile (Scheme 3.1, complex 13)

A mixture of 2-hydroxybenzothiazole (**12**, 1.00 g, 6.6 mmol) and 3-nitrophthalonitrile (**1**, 1.14 g, 6.6 mmol) in DMF (10 ml) was stirred under argon atmosphere at room temperature. K₂CO₃ (10.07 g, 72.4 mmol) was added portion-wise to the reaction mixture which was then stirred at room temperature for 48 hours. The reaction mixture was subsequently poured into ice water. The formed precipitate was filtered off, and washed with water and methanol. The crude product was recrystallized from an ethanol: water (1:1) mixture and dried *in vacuo*.

Yield: 0.95 g (52%). FT-IR (ν_{max}/cm^{-1}): 3078(Ar-CH), 2961, 2834 (CH), 2240 (CN), 1579 (C-C), 1475, 1456, 1442, 1383, 1325, 1277 (C-O-C), 740 (C-S-C). ¹H NMR (DMF-

d_7): 9.12 (1H, d, Ar-H), 8.97 (1H, d, Ar-H), 8.81 (1H, d, Ar-H), 8.67-8.57 (2H, m, Ar-H), 8.29 (1H, m, Ar-H), 7.76 (1H, d, Ar-H). Anal. $C_{15}H_7N_3OS$: Calc: C, 64.97; H, 2.54; N, 15.15; S, 11.56. Found: C, 63.66; H, 2.752; N, 16.61; S, 11.32.

2.4.1.2 3-(Carbazol-2-yloxy)phthalonitrile (Scheme 3.1, complex 15)

The synthesis of **15** was the same as for **13**, except 2-hydroxycarbazole (**14**, 1.21 g, 6.6 mmol) was employed. The other reagents, as well as reaction conditions were kept the same.

Yield: 1.2 g (59 %). FT-IR (ν_{max}/cm^{-1}): 3070 (Ar-CH), 2953, 2826 (CH), 2225 (CN), 1578 (C C), 1466, 1446, 1422, 1375 (C-O-C), 1011, 997, 954, 921, 782. ^1H NMR (DMSO- d_6): 11.46 (1H, s, NH), 8.24-8.22 (1H, d, Ar-H), 8.16-8.14 (1H, d, Ar-H), 7.85-7.78 (2H, m, Ar-H), 7.53 (1H, d, Ar-H), 7.43 (1H, t, Ar-H), 7.29 (1H, s, Ar-H), 7.28 (1H, d, Ar-H), 7.20 (1H, t, Ar-H), 7.03 (1H, d, Ar-H). Anal. $C_{20}H_{11}N_3O$: Calc: C, 77.66; H, 3.58; N, 13.58;. Found: C, 76.87; H, 4.213; N, 13.07.

2.4.1.3 4-(9H-Carbazol-2-yloxy)-phthalonitrile (Scheme 3.1, complex 16)

The synthesis of **16** was the same as for **15**, except 4-nitrophthalonitrile (**2**) was used instead of 3-nitrophthalonitrile (**1**). The amounts of reagents as well as reaction conditions were kept the same.

Yield: 1.46 g (70 %). FT-IR/ cm^{-1} : 3080 (Ar-CH), 2964, 2840 (CH), 2223 (CN), 1571 (C C), 1469, 1448, 1442, 1377, 1310, (C-O-C), 1004, 999, 962, 919, 784, 693. ^1H NMR (DMSO- d_6): 11.46 (1H, s, NH), 8.27 (1H, J = 8.4 Hz, d, Ar-H), 8.19 (1H, J = 7.8 Hz, d, Ar-H), 8.12 (1H, J = 8.8 Hz, d, Ar-H), 7.82 (1H, J = 9.4, 2.5 Hz, dd, Ar-H), 7.57 (1H, J = 8.1 Hz, d, Ar-H), 7.50 (1H, J =8.8 Hz, dd, Ar-H), 7.43 (2H, m, Ar-H), 7.22 (1H, J =7.4, t, Ar-H), 7.03 (1H, J =8.4 Hz, dd, Ar-H). Anal. $\text{C}_{20}\text{H}_{11}\text{N}_3\text{O}$: Calc: C, 77.66; H, 3.58; N, 13.58; Found: C, 77.87; H, 3.313; N, 13.77

Synthesis of **18** from **17** has been reported before, **Scheme 3.1** [200].

2.4.2 Preparation of phthalocyanines

2.4.2.1 2,(3)- Tetra (carbazol-2-yloxy)phthalocyaninato zinc (II) (Scheme 3.2, **7a**)

A mixture of zinc (II) acetate dihydrate (0.25 g, 1.1 mmol), **16** (1.36g, 4.4 mmol), DBU (3 drops) and 1-pentanol (10 mL) was stirred at 160 °C for 5 h under nitrogen atmosphere. After cooling, the solution was mixed with methanol. The green solid product was precipitated and collected by centrifuging at 5100 rpm for 10 min. The crude product was washed with ethanol, acetone, n-hexane and diethyl ether. The green product was further purified by passing through silica gel using THF as the eluent.

Yield: 0.86 g (53 %). UV/vis (DMF): λ_{max} nm (log ϵ); 680 (5.11), 612 (4.32), 362 (4.52).

FT-IR: (ν_{max} / cm^{-1}): 3200 (NH), 3161 (Ar-H), 1576, 1077, 1110 (C-O-C), 1559 (C=N), 1412

^1H NMR ($\text{DMSO-}d_6$): δ ppm 11.46-11.25 (4H, b, NH), 9.46-9.32 (2H, b, Ar-H), 9.29-9.25 (1H, b, Ar-H), 9.00-8.92 (3H, b, Ar-H), 8.39-8.11 (8H, b, Ar-H), 7.95-7.69 (4H, b, Ar-H), 7.65-7.17 (22H, b, Ar-H). Anal. $\text{C}_{80}\text{H}_{44}\text{N}_{12}\text{O}_4\text{Zn}$: Calc.: C, 73.76; H, 3.40; N, 12.90; Found: C, 73.06; H, 3.79; N, 12.88. MALDI TOF-MS (m/z): Calculated 1301; Found: 1302 $[\text{M}+\text{H}]^+$.

2.4.2.2 1,(4)- Tetra (carbazol-2-yloxy)phthalocyaninato zinc (II) (Scheme 3.2, 7b)

Synthesis and purification was as outlined for **7a**, except compound **15** was employed instead of **16**.

Yield: 0.86 g (53 %). UV/vis (DMSO): λ_{max} nm ($\log \epsilon$); 700 (5.12), 630 (4.59), FT-IR: ($\nu_{\text{max}}/\text{cm}^{-1}$): 3200 (NH), 3148 (Ar-H), 1567, 1077, 1090 (C-O-C), 1551 (C=N), 1404 (C=C). Elemental analysis $\text{C}_{80}\text{H}_{44}\text{N}_{12}\text{O}_4\text{Zn}$: Calculated: C, 73.76; H, 3.40; N, 12.90; Found: C, 73.06; H, 3.79; N, 12.88. ^1H NMR ($\text{DMSO-}d_6$): 11.46 (4H, s, NH), 8.00-7.99 (4H, d, $J = 8.4$ Hz, Ar-H), 7.93-7.92 (4H, d, $J = 7.7$ Hz, Ar-H), 7.56-7.54 (4H, t, $J = 7.8$ Hz, Ar-H), 7.34-7.31 (8H, m Ar-H), 7.18-7.15 (4H, t, $J = 7.6$ Hz, Ar-H), 7.10 (4H, s, Ar-H), 7.00-6.96 (8H, m, Ar-H), 6.79-6.78 (4H, dd, Ar-H). MALDI TOF-MS (m/z): Calculated 1301; Found: 1302 $[\text{M}+\text{H}]^+$

2.4.2.3 1,4-Tetrakis[2-dioxa-8-azaspiro[4.5]dec-8-yl)ethoxy]phthalocyanine tetraiodide (Scheme 3.3, 8)

Synthesis of **19** has been reported [200]. Synthesis of **8** was achieved by refluxing **19** (0.05 g, 0.0274 mmol) in chloroform (2 mL). Excess iodomethane (4.56 g, 32 mmol) was added drop-wise, and the reaction allowed to continue for 12 hours. After this time, the now water soluble complex **8** was sonicated and centrifuged at 31000 rpm for 5 min. This was followed by successive washing with hexane, ethyl acetate and chloroform, and the product was dried at 60 °C.

Yield: (0.04 g, 80%) (FTIR $\nu_{\max}/\text{cm}^{-1}$): 3311 (Ar-CH), 2987–2864 (C-H), 1579–1708 (C-C), 1668 (C=C), 1483 (C-H bend) 1331 (C-O) 1269 (C-N) 744–896 (Ar-CH bending). ^1H NMR (600 MHz, D_2O) δ ppm: 7.73–7.65 (b, 3H, Ar-H), 7.33–6.93 (9H, m, Ar-H), 3.40–3.31 (m, 3H, -CH₂), 3.30–3.12 (b, 30H, -CH₂), 2.98–2.60 (b, 12H, -CH₃), 2.25–1.95 (b, 31H, -CH₂) 2.25–1.95 (b, 2H, NH). Elemental analysis: calcd. (%) for $\text{C}_{72}\text{H}_{90}\text{N}_{12}\text{O}_{12}$: C 47.43, H 4.98, N 9.22, Found: C 48.36, H 3.71, N 9.49. MALDI-TOF MS: m/z : Found: 1314 [$\text{M}-4\text{I}$]⁺.

2.4.2.4 1,(4)-Tetra (2-benzothiazol-2-yloxy) phthalocyaninato zinc (II) (Scheme 3.2, complex 9)

Synthesis and purification was as outlined for **7a**, except **13** (0.26 g, 1 mmol) was employed instead of **16**.

Yield: 0.215 g (10%). UV/vis (DMSO): $\lambda_{\max}\text{nm}$ (log ϵ); 672 (5.29), 606 (4.59), 337 (4.79) FT-IR: ($\nu_{\max}/\text{cm}^{-1}$): 3018 (Ar-H), 1598 (C=C), C=N (1560), 1288, 1120 (C-O-C), 940 (C-S-C), 730, 714, 760, 830 (C-H). Anal. $\text{C}_{60}\text{H}_{28}\text{N}_{12}\text{S}_4\text{O}_4\text{Zn}$: Calc: C, 61.35; H, 2.39; N, 14.32;

S, 10.91. Found: C, 61.50; H, 2.09; N, 13.96; S, 10.34. ^1H NMR ($\text{DMF-}d_7$): 10.00-9.75 (4H, m, Ar-H), 9.70-9.45 (4H, m, Ar-H), 8.85-8.70 (8H, m Ar-H), 8.68-8.60 (6H, m, Ar-H), 8.55-8.40 (6H, m, Ar-H). MALDI TOF-MS: Calculated: 1174; Found: 1181 $[\text{M}+7\text{H}]^+$

2.4.3 Synthesis of ZnTAPc (10)-FA conjugate (Scheme 3.4)

The conjugation of folic acid with zinc tetraaminophthalocyanine to form the **10**-FA conjugate was achieved as follows: folic acid (0.014 g, 0.031 mmol), DCC (0.019 mg, 0.094 mmol) and NHS (3.62 g, 0.031 mmol) were stirred in the dark in a solvent mixture of 8 mL of DMSO and 0.5 mL of pyridine for 16 hours. Complex **10** (5 mg, 7.86 μmol), dissolved in DMSO, was added drop-wise into the mixture, the reaction was further stirred in the dark, at room temperature for 24 hours. The solution was precipitated out with CH_2Cl_2 /hexane to yield the conjugate. The formed conjugate was purified by repetitive dissolution in DMSO and precipitating out with CH_2Cl_2 /hexane.

Yield: 67%. UV-Vis (DMSO): λ_{max} nm ($\log \epsilon$), 695 (5.4), 354 (5.6) and 286 (5.8). MALDI TOF MS (m/z): Calculated 1059 g/mol Found: 1052 (M-7H). IR: (cm^{-1}): 3236 (N-H str.), 1689 C=O amide, 1607 (N-H bend), 1140 C-C str., 1041 (C-N str.) ^1H -NMR (400 MHz, $\text{DMSO-}d_6$): δ /ppm : 4.5 (m, FA), 6.5 (m, FA), 6.6-6.7 (br, Pc-H), 7.6-7.7 (br, Pc-H,FA), 8.6 (br, Pc-H, FA).

2.4.4 Tris {9(10),16(17),23(24)-4-(2-mercaptopyridine)-2-(4-carboxyphenoxy) phthalocyaninato} magnesium (II)-polylysine conjugate (Scheme 3.5)

The conjugate of polylysine and tris {9(10),16(17),23(24)-4-(2-mercaptopyridine)-2-(4-carboxyphenoxy) phthalocyaninato} magnesium (II) (**11**), was prepared and purified as follows: **11** (25 mg, 0.025 mmol) was activated with NHS (3.4 mg, 0.029 mmol) and DCC (2.5 mg, 0.012 mmol) for 12 hours at room temperature in DMSO as a solvent. PLL (50 mg) was added to the solution and the reaction was allowed to proceed for 24 hours. The product was precipitated out with chloroform and purified by dialysis and size exclusion chromatograph (Sephacryl S-100 column) with 0.05 % TFA in 30:70 acetonitrile:water, respectively. These solvents were removed from the fraction of interest with a lyophilizer.

2.4.5 Conjugation of Pcs to NPs or MPs

2.4.5.1 Synthesis of MPc-ZnO conjugate (Scheme 3.6).

Citric acid capped zinc oxide microparticles (ZnOMPs) were synthesized as reported in literature [201,202], **Scheme 3.6**. The prepared particles are termed microparticles due to their large size. Conjugation of ZnO microparticles (ZnOMPs) with complex **7a** was achieved as follows (**Scheme 3.6**): the carboxylic acid group of the citric acid capping ligand of ZnO microparticles (0.014 g/mL) in DMSO was activated with DCC (0.019 mg, 0.094 mmol) and NHS (3.62 mg, 0.031 mmol) for 12 hours. Complex **7a** (5

mg, 0.00384 mmol) dissolved in DMSO (1 mL), was added dropwise into the mixture, and the reaction was conducted for 36 hours. The conjugate was purified by passing the reaction mixture through a Sephadex® G-25 column with DMSO as an eluent.

2.4.5.2 Synthesis of MPc-AgNPs conjugate (Scheme 3.7).

Citric acid capped silver nanoparticles (AgNPs) were prepared following reported method [4]. The conjugation was achieved by a well documented method, but with slight modification [203]. Citric acid capped silver nanoparticle were concentrated by centrifugation and dried at room temperature. AgNPs (5 mg) and **7a** (2 mg, 0.0015 mmol), **7b** (2 mg, 0.0015 mmol), **8** (2.8 mg, 0.0015 mmol) or **10** (1 mg, 0.0015 mmol) were dissolved in 3 mL of DMSO, and stirred at room temperature for 96 hours. The conjugate was purified by passing the reaction mixture through a Sephadex® G-25 column with DMSO as an eluent.

2.4.6 Preparation of electrospun fibers

The electrospinning technique (with or without MPc complexes) has been described before [153, 159, 160, 204]. Electrospun nanofibers were prepared as follows: A solution containing 2.5 g of polystyrene (PS), 5 mg of sample (**7a**, **7a**-ZnOMPs, **7a**-AgNPs, **7b**, **7b**-AgNPs, ZnOMPs, or AgNPs) and 10 mL of DMF/THF (4:1) was stirred for 24 hours to produce a homogeneous solution. The solution was then placed in a syringe fitted with a capillary needle. A potential difference of 20 kV was applied between the anode and cathode. The distance between the cathode (static fiber collection point) and

anode (tip of capillary needle) was 15 cm. The pump rate was maintained at 0.05 mL/h.

2.4.7 Preparation of chitosan beads

Preparation of beads was achieved following the coacervation precipitation method reported previously [205]. Briefly, for the preparation of **10**/chitosan, a mixture of chitosan (0.2 g) and **10** (2 mg, 0.029 mmol) in 2 % acetic acid (10 mL) was stirred continuously for 24 hours. After complete mixing, the homogeneous solution was forced through a syringe needle into a beaker containing 2 M NaOH solution, with the beads forming immediately. These beads were stored in the alkaline solution for 24 hours, and then washed several times with distilled water until the pH of water was neutral. For the preparation of **10**-AgNPs/chitosan beads, purified **10**-AgNPs (2 mL) and 0.2 g of chitosan were mixed in 10 mL of 2% acetic acid. The mixture was stirred at room temperature, and beads were prepared as outlined above. Unfunctionalized chitosan beads were also prepared using a similar method.

CHAPTER 3

SYNTHESIS & CHARACTERIZATION

This chapter details the synthetic methods employed as well as the characterization of phthalocyanines and phthalocyanine conjugates. The methods used for the characterization of the electrospun fibers and microbeads are also presented.

PUBLICATIONS

Publications in peer-reviewed journals that resulted from the results presented in this thesis are listed below. These articles are not referenced in this thesis:

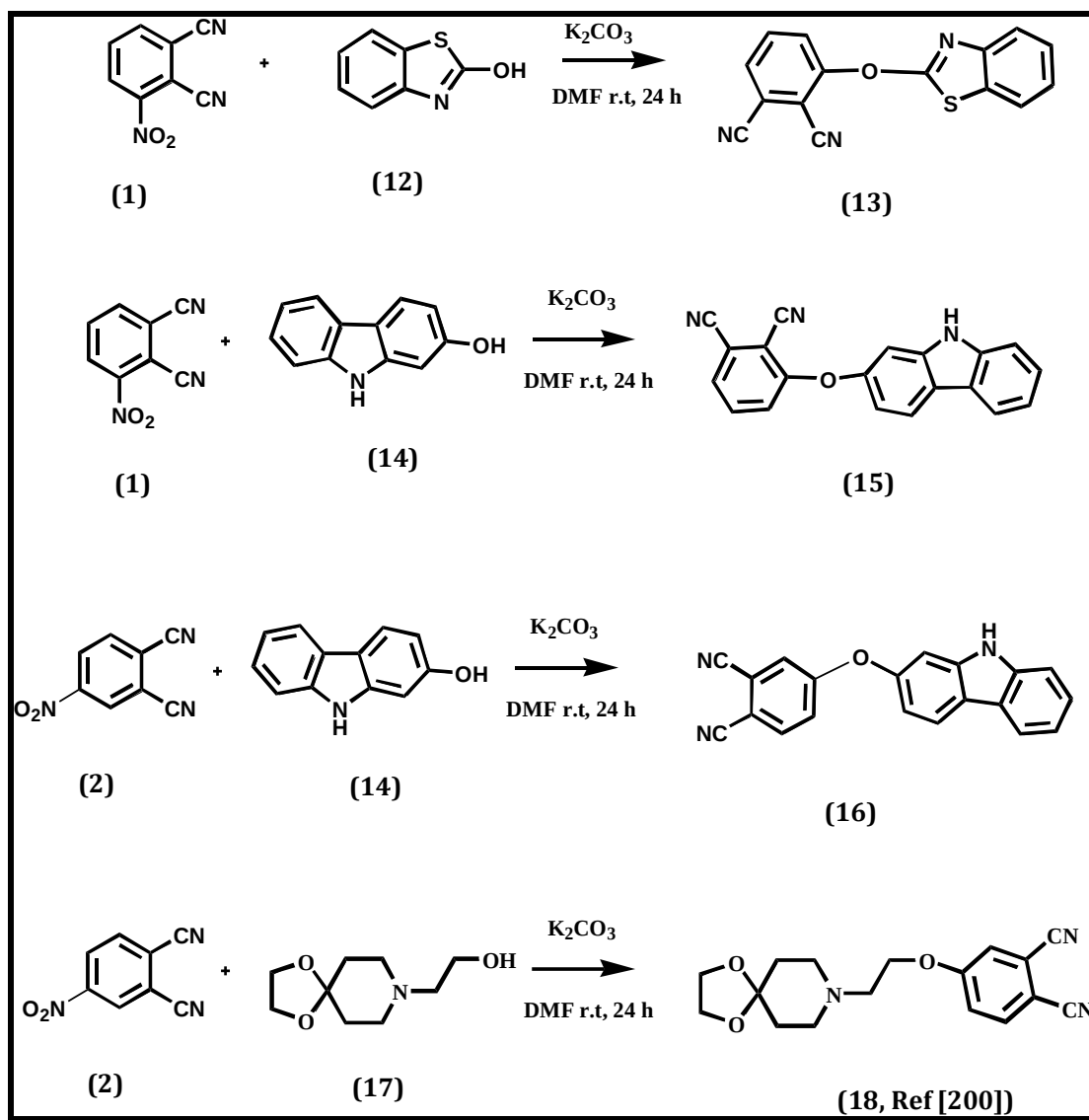
1. P. Khoza, Edith Antunes, Ji-Yao Chen, Tebello Nyokong, Synthesis and photophysicochemical studies of a water soluble conjugate between folic acid and zinc tetraaminophthalocyanine, *J. Luminescence* **134** (2013) 784-790.
2. P. Khoza, E. Antunes, T. Nyokong, Synthesis, photophysicochemical properties and biological activity of benzothiazole or carbazole derivatized phthalocyanine *Polyhedron* **61** (2013) 119-125.
3. P. Khoza, E. Antunes, T. Nyokong, Photophysicochemical behaviour of carbazole derivatized phthalocyanine in the presence of ZnO microparticles and when embedded in electrospun fibers, *Dyes and Pigments* **104** (2014) 57-66.
4. P. Khoza, E. Antunes, T. Nyokong, Photocatalytic behaviour of phthalocyanine-silver nanoparticle conjugates supported on polystyrene fibers, *Journal of Molecular Catalysis A: Chemical* **395** (2014) 34-41.
5. P. Khoza, T. Nyokong, Photocatalysis of zinc tetraamino phthalocyanine-silver nanoparticles immobilized on chitosan beads, *Journal of Molecular Catalysis A: Chemical* **399** (2015) 25-32.

6. P. Khoza, T. Nyokong, Visible light transformation of Rhodamine 6G using tetracarbazole zinc phthalocyanine when embedded in electrospun fibres and in the presence of ZnO and Ag particles, **Journal of Coordination Chemistry**, doi: 10.1080/00958972.2015.1013944.
7. P. Khoza, Ivy Ndhundhuma, Aletta Karsten, Tebello Nyokong, Photodynamic therapy activity of phthalocyanine-silver nanoparticles on melanoma cancer cells, **Medical Photonics**, *accepted with revision*

3.1 Synthesis and characterization of MPcs and MPcs conjugated to folic acid or polylysine

3.1.1 Synthesis and characterization of Pcs (7a, 7b, 8 and 9)

The synthesis of **10**, **11**, **18** and **19** have been reported before, hence, were not included in the experimental section [196,197,200]. Complexes **7a**, **7b**, **8** and **9** are new and are therefore discussed in this section. **Scheme 3.1** gives the synthetic pathways for the preparation of the phthalonitriles used to prepare the phthalocyanines reported in this work. The substituted phthalonitriles were attained using well-established methodologies [58,59]. The electron-withdrawing capability of the dinitrile functionalities makes **1** and **2** susceptible to nucleophilic attack. The base catalyzed nucleophilic substitution of the nitro groups (**1**, **2**) was performed in dry DMF, at room temperature and under inert atmosphere. The ^1H -NMR spectra confirmed the purity of the phthalonitriles by the observation of the proton peaks in their respective regions, as described in the experimental section. The phthalonitriles were also characterized by FT-IR. A distinct $\text{C}\equiv\text{N}$ stretch, between 2223 cm^{-1} and 2240 cm^{-1} , was observed for all phthalonitriles.

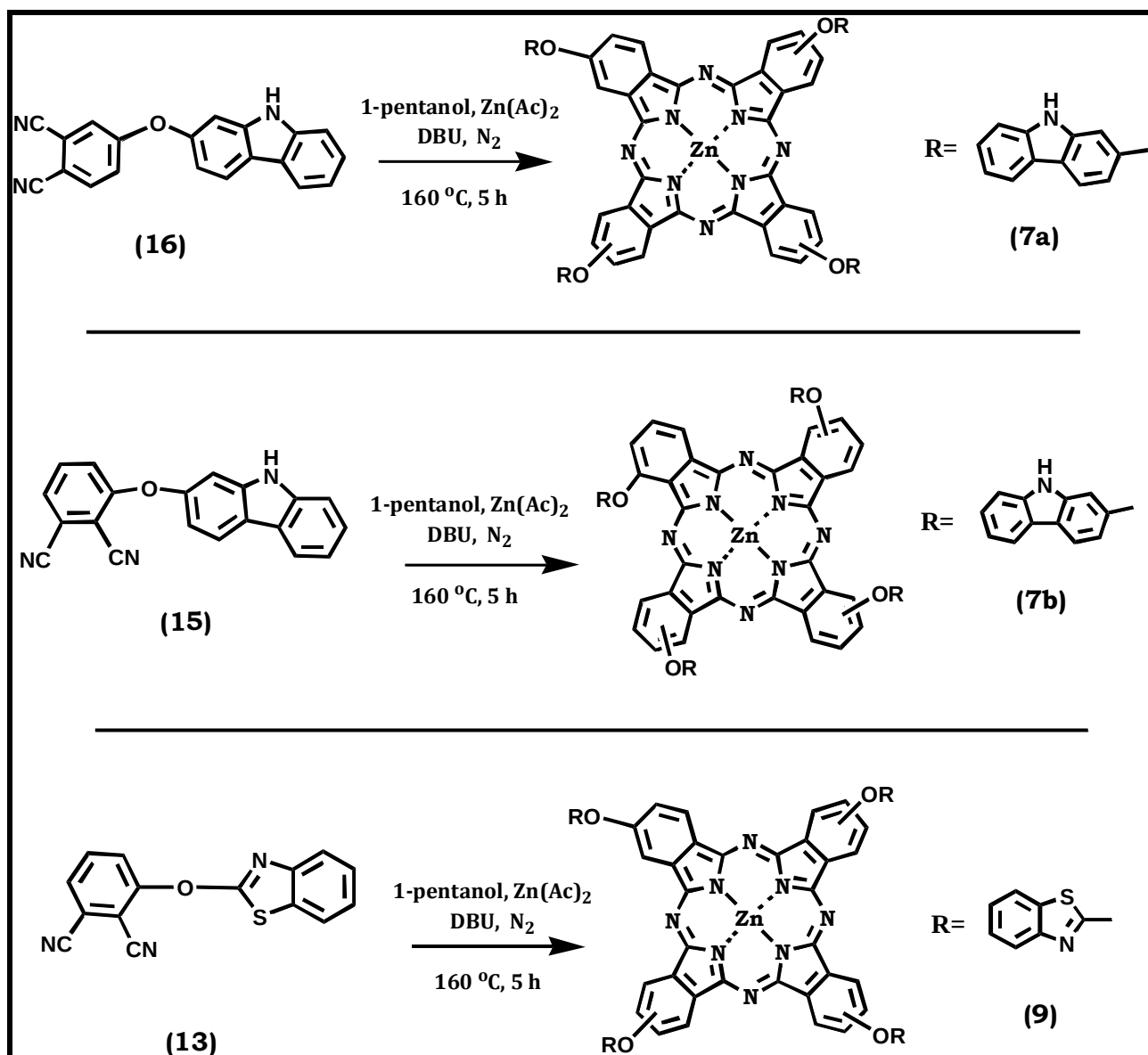


Scheme 3.1: Synthetic routes to the phthalonitriles reported in this work.

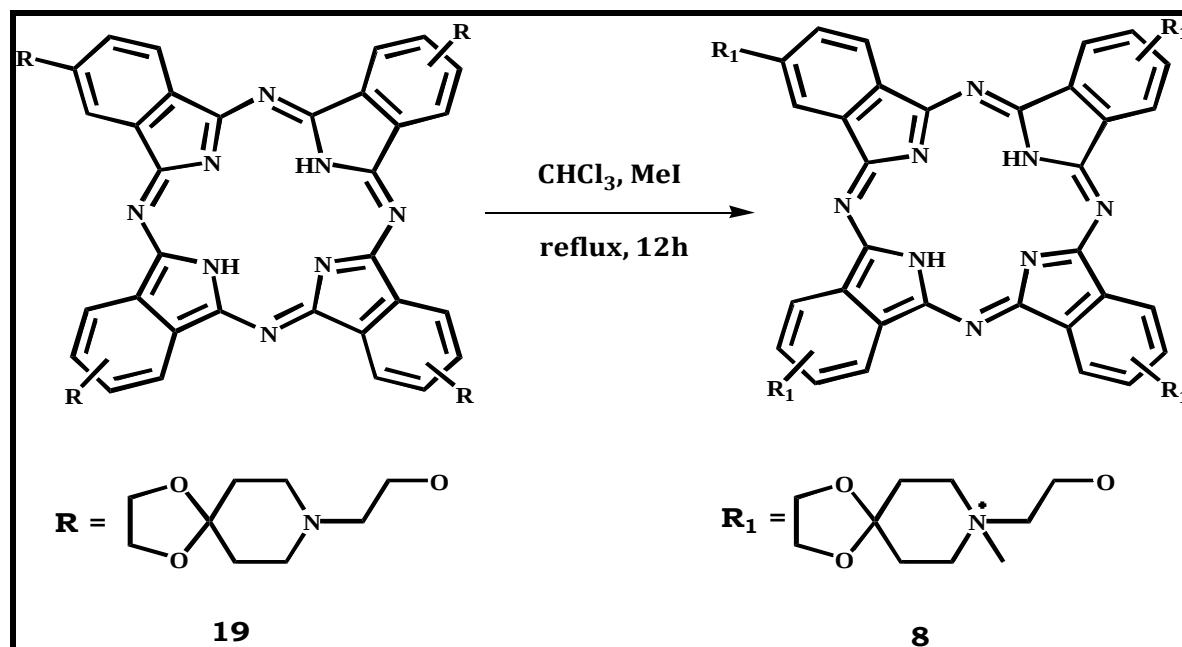
Phthalocyanines (**7a**, **7b**, **8** and **9**) were prepared by the cyclotetramerization of the carbazole, ketal and benzothiazole phthalonitriles (**Schemes 3.2** and **3.3**). Cyclization of the phthalonitriles was confirmed by the disappearance of the CN peak, between 2223 and 2240 cm^{-1} , in the FT-IR spectra. The formed compounds were further characterized using elemental analysis. It has been reported that for large Pc molecules, as in this work, elemental analysis often gives unsatisfactory results [206]. However, the results obtained were satisfactory, and were consistent with the

proposed structures, with %C differences between experimental and theoretical values less than one. The compounds were found pure by ^1H NMR, with the substituents and ring protons observed in their expected, respective regions. The presence of isomers as well as phthalocyanine aggregation at concentrations typically used for NMR measurements lead to broadening of the aromatic signals. For compound **9**, the broad signals ranging from 8.68-8.60 and 8.55-8.40 ppm were attributed to the protons from the ring and integrated to 6 protons each. The three broad signals assigned to the protons of the benzothiazole substituent were found to reside between 10.00 and 8.70 ppm.

For complex **7a**, the aromatic signals of the Pc ring and the carbazole substituent were observed between 7.17 and 9.46 ppm. There were differences between the ^1H NMR spectrum of **7a** and **7b**. For complex **7b**, signals at 7.93-7.92 ppm and 8.00-7.99 ppm integrated to 4 protons each; these were attributed to the protons of the Pc ring. A signal at 7.00-6.96 ppm integrated to 8 protons and was assigned to both the Pc ring and carbazole substituent. The other signals in the aromatic region were assigned to the protons of the substituent ring system. Similarly to **7a**, the NH proton was observed at 11.49 ppm.

*Scheme 3.2: Synthesis of 7a, 7b and 9.*

The ^1H NMR spectrum of compound **8** showed aromatic signals between 7.73–6.93 ppm that were due to the protons of the phthalocyanine ring. Aliphatic CH_2 and CH_3 protons of the substituent were observed between 3.40 and 1.95 ppm.



Scheme 3.3 Synthesis of complex 8.

MPc complexes have been observed to degrade with molecular ion peaks $[\text{M}]^+$, $[\text{M}+n\text{H}]^+$ or $[\text{M}-n\text{H}]^+$ [207]. Some matrices intensify certain processes. However, the mass spectra of complex **7a** and **7b** showed a clear molecular ion peak, **Figure 3.1**, unlike complex **8** and **9** which gave complicated mass spectra, this is due to the different fragmentation patterns explained above.

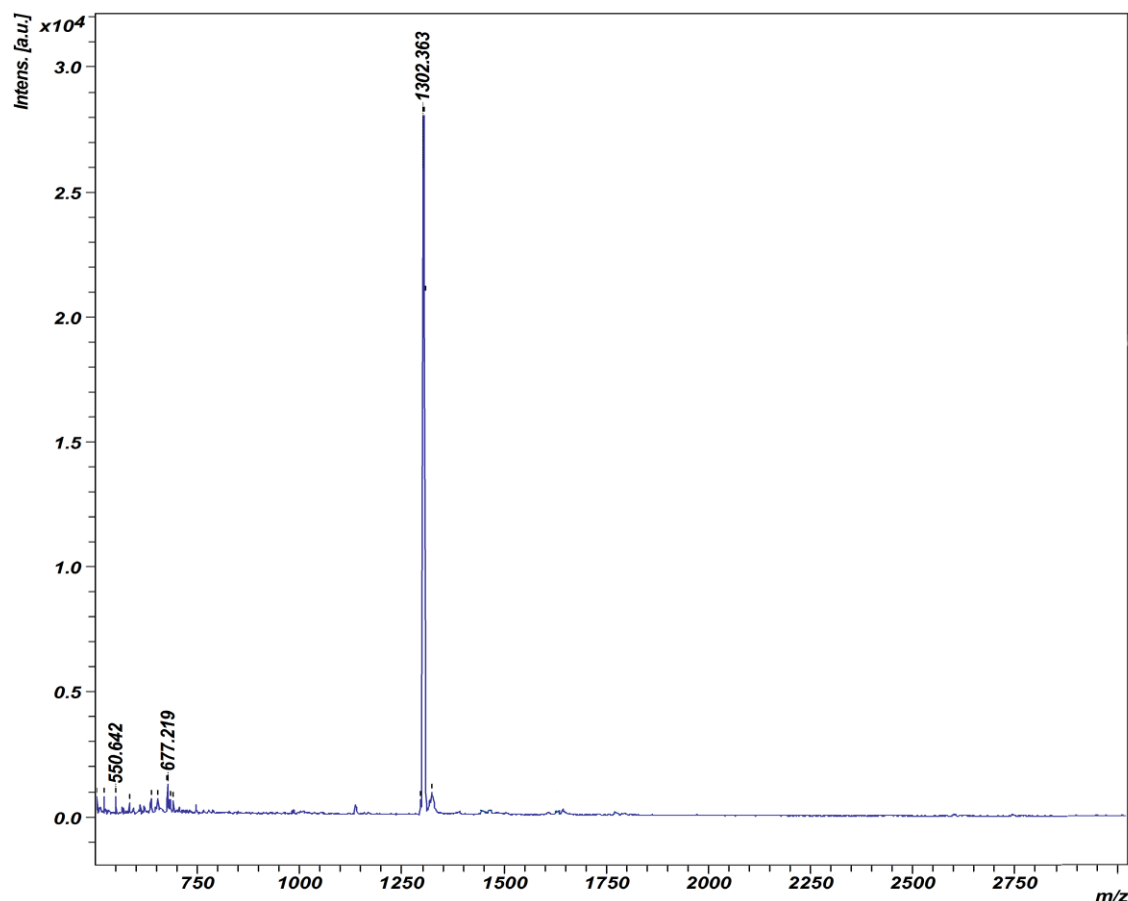
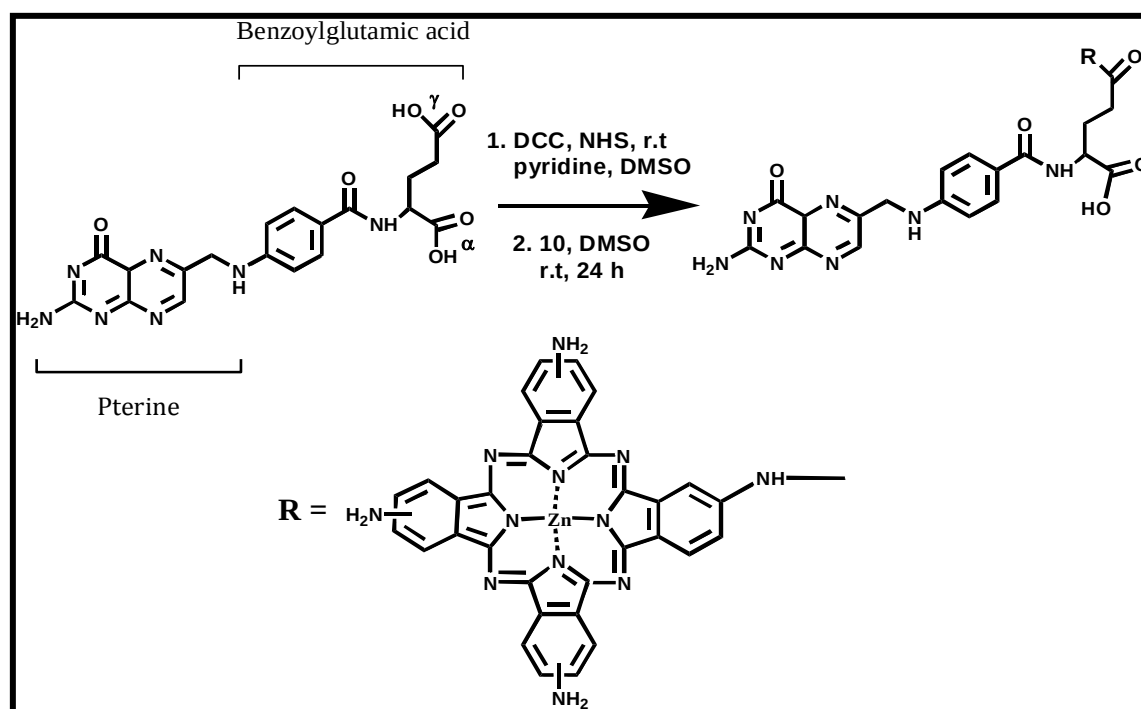


Figure 3.1: Mass spectrum of complex 7a

3.1.2 Synthesis and characterization of 10-FA

Scheme 3.4 shows the synthetic route for zinc tetraaminophthalocyanine and its folic acid conjugate (**10-FA**). The activation of the carboxylic acid group on FA was performed using DCC/NHS in the presence of basic solvents (DMSO and pyridine). Complex **10** was chosen for linking to FA due to the presence of NH₂ groups which would allow for amide bond formation with the COOH functional group of FA. The NH₂ of FA can also be used to form a link with the COOH of **11**. However, in this

work, linkage through COOH of FA was chosen as reports have shown that the pterine moiety of folic acid is easily recognised by FA receptors [121]. Upon conjugating **10** to FA, there are many possible structures that could form; these include the coordination of 1, 2, 3 or 4 folic acid units to **10**. Folic acid has two carboxylic acids which can act as points of attachment during the formation of the conjugate. However, it is well documented that upon activation, the γ carboxylic acid is the easiest to activate, hence, the major product obtained is through conjugation with the γ carboxylic [120,208]. The mass spectrum of complex **10**-FA shows a molecular ion peak at 1052 amu corresponding to a 1:1 ratio of Pc: FA complex. Even though more ratios were possible, MALDI TOF MS showed 1:1 ratio between folic acid and **10**. Complex **10** is insoluble in aqueous media, but when chemically linked with FA, solubility in aqueous media is induced.



*Scheme 3.4: Conjugation of FA to **10** (ZnTAPc) via the condensation reaction between the activated folic acid and **10**.*

FTIR was employed to confirm the presence of the amide bond in **10**-FA. This was, however, complicated by the fact that FA has an amide bond. The FTIR spectrum of **10** is shown in **Figure 3.2 (iii)**. The secondary amine stretching vibrations are found at 3324 and 3192 cm^{-1} , and N-H bending is observed at 1600 cm^{-1} . The conjugate, **10**-FA, exhibits a peak at 1685 cm^{-1} **Figure 3.2 (i)**, the peak corresponds the carbonyl peak for folic acid which appeared at 1689 cm^{-1} , the shift indirectly confirms coordination. There were no significant shifts in the IR peaks when FA was mixed with **10** without the addition of the coupling agents, hence, this further confirms the formation of the conjugate.

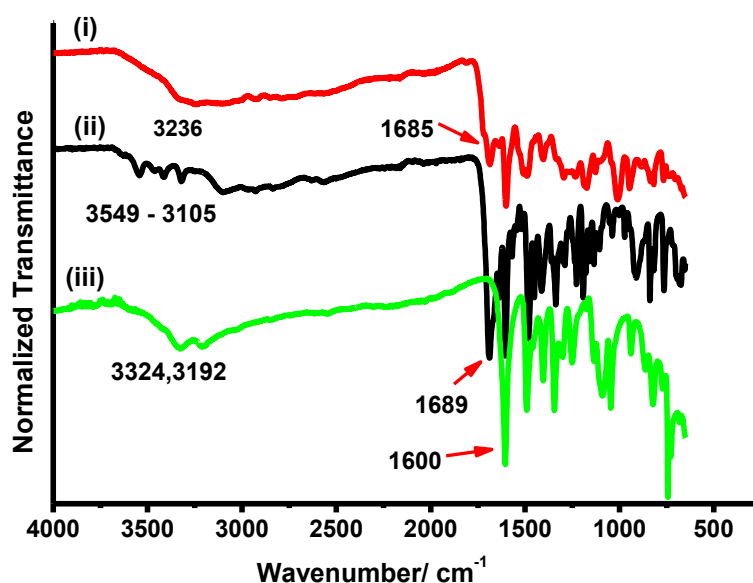


Figure 3.2: FTIR spectra of ZnTAPcFA (**10**-FA) (i), folic acid (ii) and ZnTAPc (**10**) (iii).

The ^1H NMR of folic acid is well known, therefore, only the characteristic peaks are discussed herein. For unconjugated folic acid, the aromatic signals were observed at 7.7 and 6.5 ppm, and integrated for two protons each. These protons reside on the

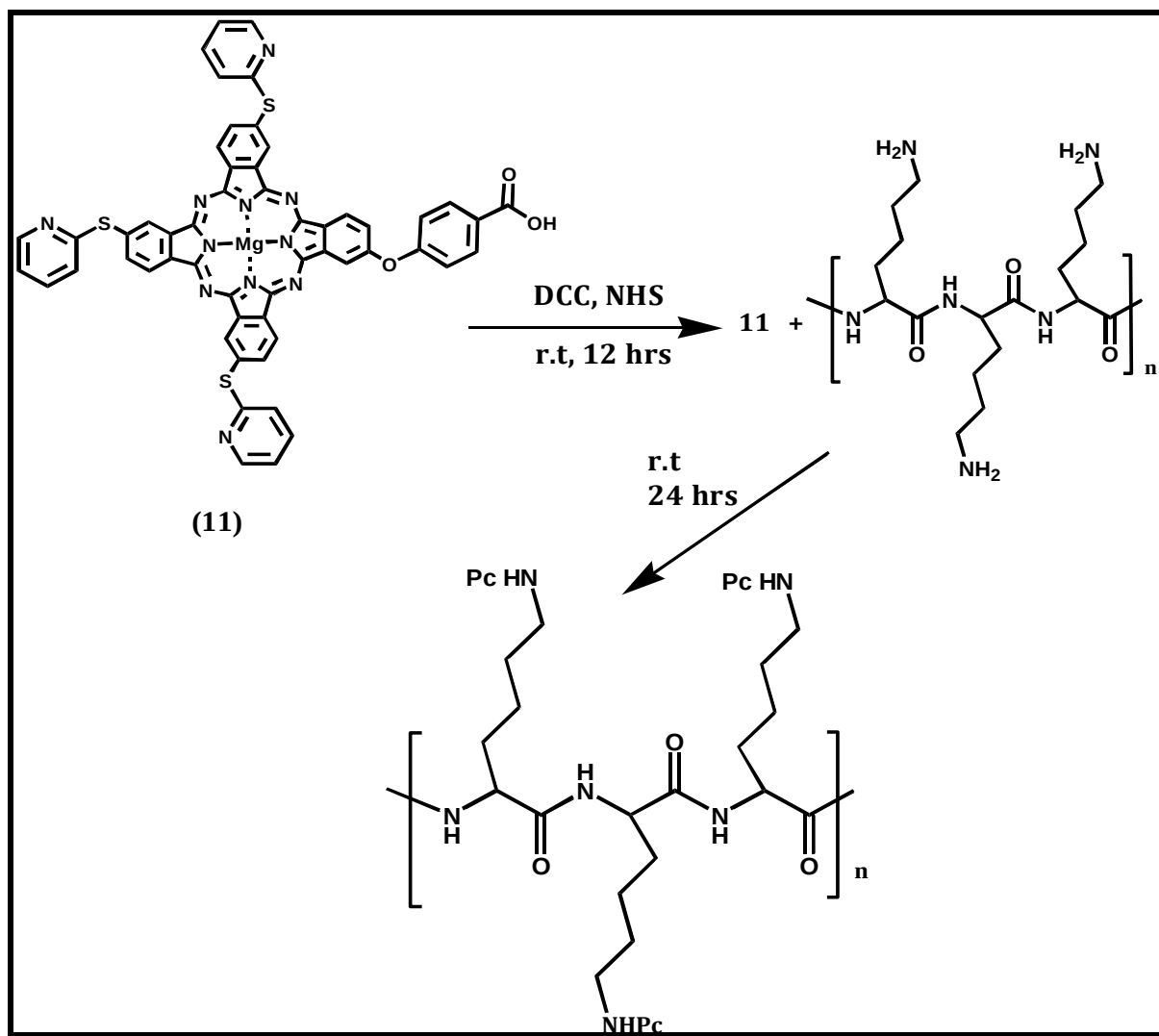
benzene ring of folic acid. Unconjugated complex **10** was found pure by ^1H NMR and all the peaks were well resolved. The aromatic signals at 8.92 (d, 4H), 8.42 (d, 4H) and 7.39 (s, 4H) were assigned to the ring of **10**. The broad signal at 6.23 ppm, which integrates to 8 protons, was assigned to the amino protons. The aromatic region of **10**-FA conjugate showed complex patterns due to the overlapping of signals from **10** and folic acid. Upon formation of a chemical link between folic acid and Pc, there is a significant shift in the signals from both the Pc and folic acid. The shifting and subsequent overlap indicate a change in the chemical environment as a result of the conjugate formation.

In addition, conjugating the Pc to folic acid is expected to result in the disappearance of the amine peaks from the Pc but only if an amide bond is formed with all four amino groups of the Pc. The ^1H NMR of **10**-FA conjugate shows a broad signal in the range 6.6 – 6.7 ppm due to Pc's amino protons and FA protons. The broadened signals in the region between 7.6 - 7.7 ppm are due to overlap between the Pc ring protons and FA peaks. The benzene ring protons which were at 6.5 and 7.7 ppm in unconjugated folic acid are still clearly seen as broad signals (6.6 - 6.7 ppm and 7.6 - 7.7 ppm, respectively) with the same coupling constant (8.45 Hz). This indicates that the broadened signals are due to FA and **10**. It was difficult to obtain integration values due to overlapping of peaks. Nonetheless, the shifts in the NMR spectra are an indication of changes in the environment, which indicates conjugation.

3.1.3 Synthesis and characterization of **11**-PLL

Scheme 3.5 shows the synthetic pathway for complex **11** conjugated to polylysine. Complex **11** was chosen as it provides a direct point of conjugation compared to a Pc tetrasubstituted with COOH, this in turn reduces complications that arise as a result of the presence of many points of linkage to PLL. PLL has several amino groups that are capable of conjugating to **11**. After covalently linking **11** and PLL, confirmation for the formation of the conjugate was evident in the solubility of **11**-PLL in water, and through the use of thin layer chromatography (TLC).

The ^1H NMR and IR obtained for this conjugate did not give any conclusive evidence with regards to the formation of the amide bond between **11** and polylysine. The spectra was complicated by several amide bonds in polylysine, and therefore, did not confirm the conjugation unequivocally. Nonetheless, after passing the conjugate through a size exclusion column, the purity of the conjugate was confirmed with a silica based TLC plate (**Figure 3.3**), which shows the disappearance of the fluorescent **11** band after purification, **Figure 3.3B**. In addition, the conjugate was only soluble in water, while **11** was highly insoluble in water.



Scheme 3.5: Conjugation of 11 and polylysine by the activation of 11 with DCC, NHS coupling agents.

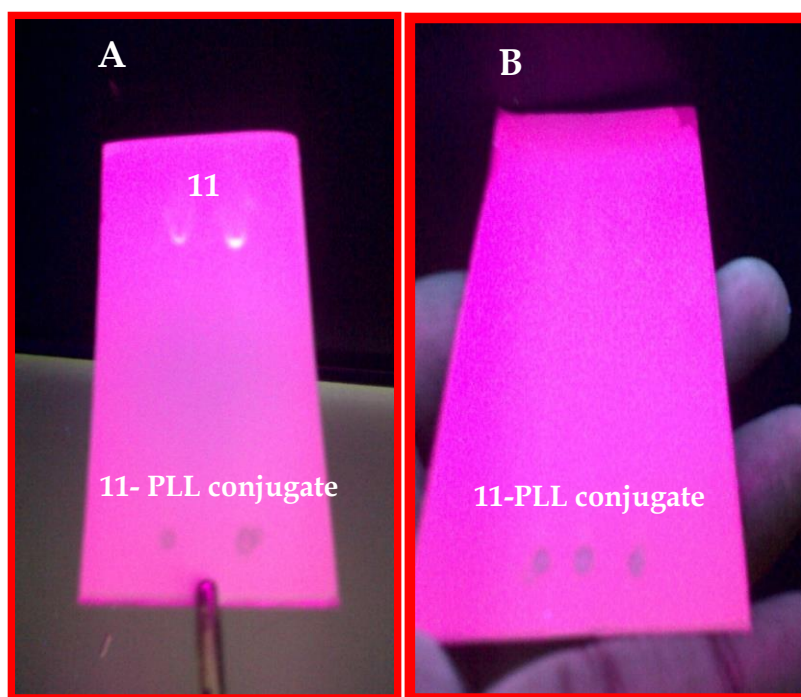


Figure 3.3: TLC plates of the conjugate prior to the separation (a) and after the purification (b) by using size exclusion. Silica TLC plates are viewed under UV light.

3.2 Ground state absorption spectra of MPcs

3.2.1 Ground state absorption spectra of Pcs (7a, 7b, 8 and 9)

The ground state absorption spectra for **7a**, **7b**, **8** and **9** showed monomeric behaviour as evidenced by narrow Q bands which is typical of phthalocyanines [70], **Figure 3.4-3.6**. **Figure 3.4** shows the absorption spectra of both non-peripherally (**7b**) and peripherally (**7a**) substituted phthalocyanines in DMSO. There is red shifting of the Q-band of **7b** compared to **7a**. The changes observed are attributed to the linear combination of the atomic orbitals (LCAO) coefficients being greater at the non-peripheral positions of the HOMO than those at the peripheral position [209-212].

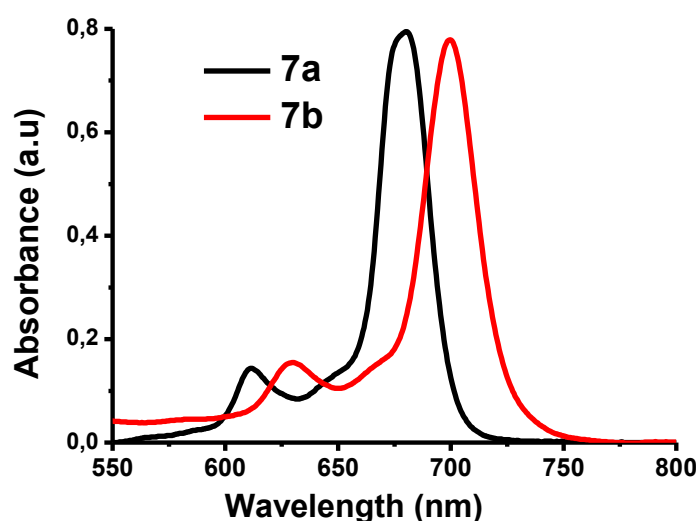


Figure 3.4. Absorption spectra of 7a and 7b in DMSO.

Generally, metallophthalocyanines are insoluble in most organic solvents, however, the introduction of substituents on the ring system increases solubility. Complexes **7a**, **7b**, **8** and **10** exhibited excellent solubility in a number of organic solvents. Significant red shifting was observed in pyridine, **Figure 3.5**, **Table 3.1**. The phthalocyanine Q band shifts to the red with an increase in the refractive index of the solvent [209], hence, red shifting is observed in pyridine which has a larger refractive index compared to the other solvents investigated. When comparing the unsubstituted ZnPc (Q band absorption at 672 nm in DMSO [82]) and the benzothiazole derivatized ZnPc (**9**), there is no significant difference in the position of the Q band. With carbazole substituted ZnPc (complex **7b**), there is a clear red shift as compared to benzothiazole derivatized Pc (**9**). It has been reported that overlap of bulky groups located at the non-peripheral positions of the ring (such as phenyl groups) causes substantial deformation of the phthalocyanine [210], this results in the splitting and the red

shifting of the Q band. Thus, the red shift in complex **7b** compared to **9** (both α substituted) could be related to the bulkiness and rigidity of the carbazole substituent (**7b**) compared to benzothiazole (**9**).

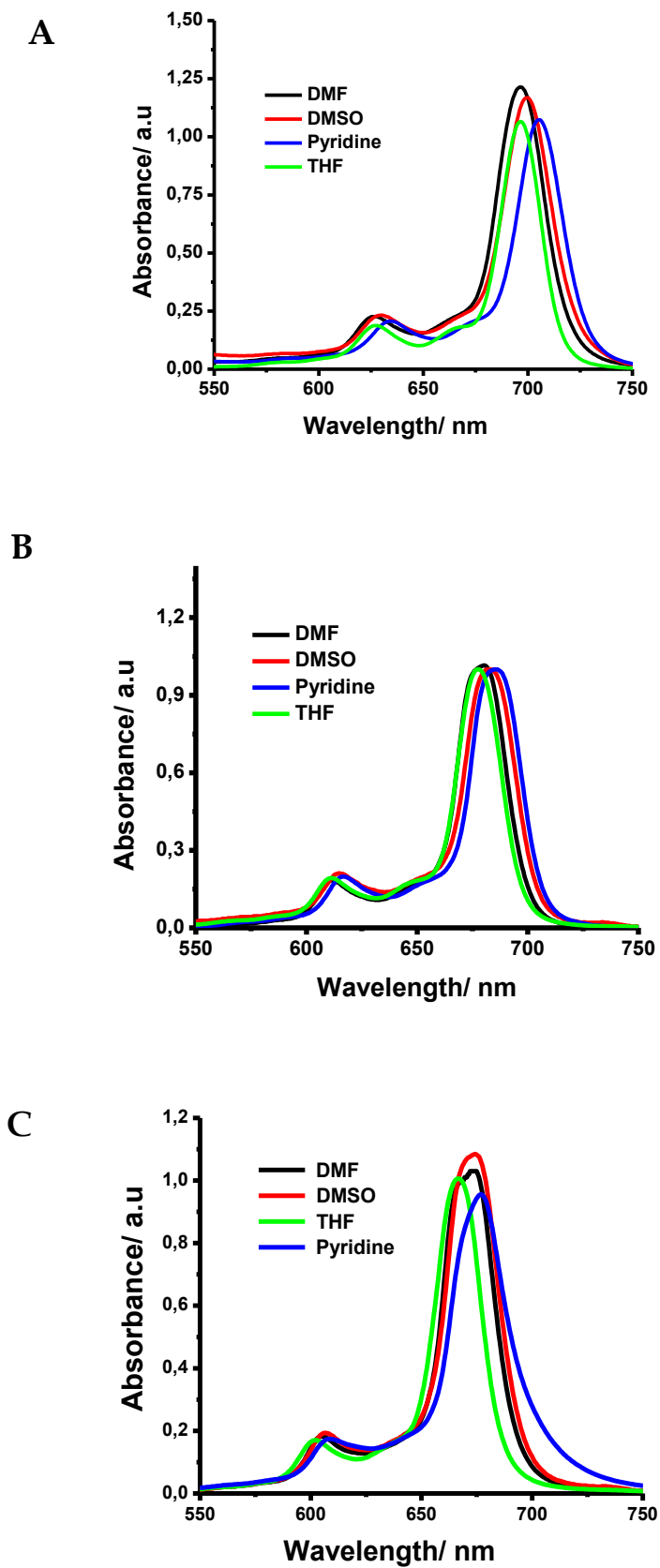


Figure 3.5: Absorption spectra of **7b** (A), **7a** (B) and **9** (C) in different organic solvents

Table 3.1: Ground state absorption spectra of 7a, 7b, 8 and 9 in different solvents.

Complex	Solvent	Q band λ_{max} (nm)
7a	DMSO	682
	DMF	680
	Pyridine	686
	THF	677
7b	DMSO	700
	DMF	694
	Pyridine	705
	THF	697
8	DMSO	676, 704
	DMF	675, 703
9	DMSO	672
	DMF	671
	Pyridine	677
	THF	669

The ground state absorption spectra of complex **8** in water and DMF are shown in **Figure 3.6**. The complex was highly aggregated in water, this is indicated by a broad absorption peak at 625 nm [70]. Although water is a very good solvent for biological applications, it has been reported that singlet oxygen generation is greatly reduced in this solvent [82]. The surfactant, sodium dodecyl sulphate (SDS) (concentrations = 3.27 mM), resulted in the disaggregation of **8**, **Figure 3.6**. The complex was not aggregated

in DMF, as evidenced by a split Q band at 670 and 704 nm. The split is typical of metal free Pcs, **Figure 1.6** [71].

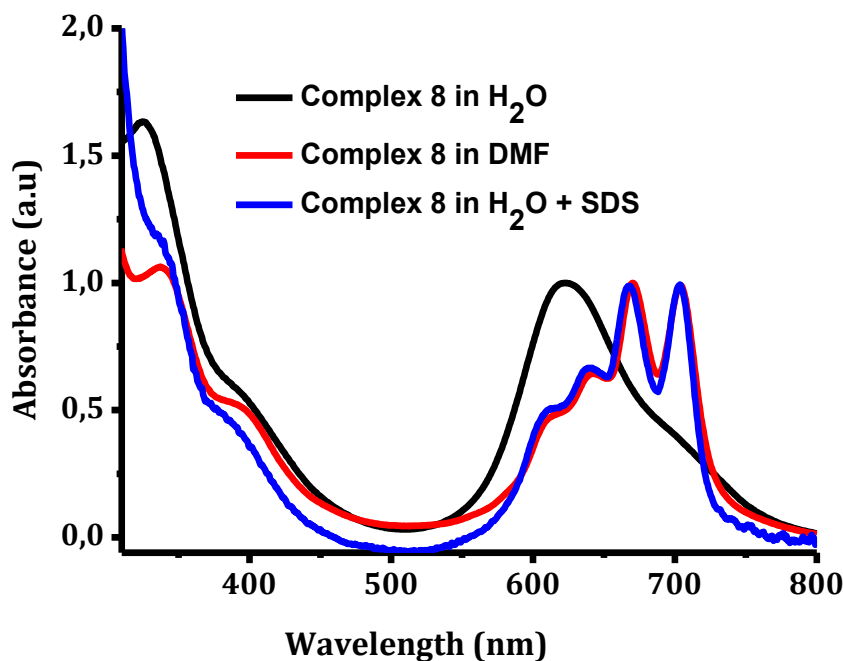


Figure 3.6: Absorption spectra of complex 8 in DMF and in water. The figure also shows the breaking of aggregates by adding the surfactant SDS.

3.2.2 Ground state absorption spectra of 10-FA and 11-PLL

Figure 3.7 shows the absorption spectra of **10**+FA mixed (a), **10**-FA covalently linked (b), folic acid (c) and **10** (d). The Q band absorption peak of zinc tetraamnimophthalocyanine appears at 715 nm in DMSO [196]. Folic acid has a typical broad band between 400 and 280 nm [213]. The conjugate formed between folic acid and **10** resulted in the blue shifting (20 nm) of the Q band of **10** from 715 nm to 695 nm (**10**-FA) in DMSO. No shifting in spectra was observed when a free base tetraphenyl

porphyrin was linked to folic acid in the presence of a linker, this was because the distance between the porphyrin and folic acid was long enough to prevent any interaction between porphyrin and folic acid [208]. In this study, covalently linking folic acid to **10** without any linker resulted in the blue shifting of the Q band. In general, the amino groups result in red shifting of the Q band due to their electron donating nature, resulting in an extension of the ring π conjugation. Upon conjugation, blue shifting resulted since one of the amino groups of **10** is now engaged upon coordination to folic acid. A smaller blue shift, of 9 nm, was also observed when folic acid was mixed with **10**, **Figure 3.7a**. Since there is no coordination, the blue shift reflects a change in the environment especially of the amino groups. The B band region shows the presence of both **10** and folic acid absorption peaks (**Figure 3.7b**). The difference in spectra between the chemically linked (**10-FA**) and mixed (**10+FA**) is an indirect way of confirming the linkage between folic acid and **10**.

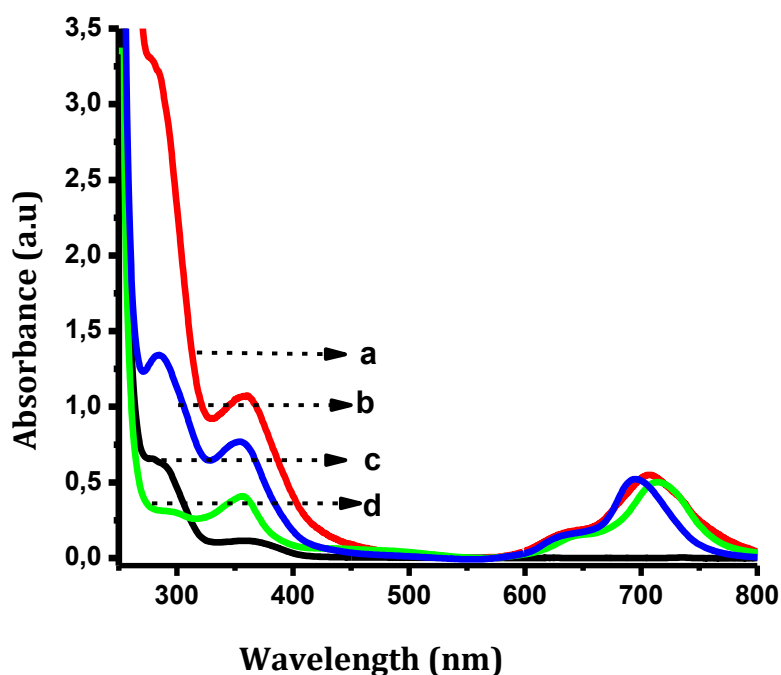


Figure 3.7: Absorption spectra of **10**+FA mixture (a), **10**-FA covalently linked (b), folic acid (c) and **10** (d) in DMSO.

By chemically linking folic acid, **10** became soluble in aqueous media; the Q band was observed at 714 nm, **Figure 3.8a**. Phthalocyanines have a tendency to form aggregates in water. Hence, the spectrum obtained in water is broad due to the formation of aggregates [70]. The Q band of the monomer peak of **10**-FA is red shifted in water (710 nm, **Figure 3.8a**) when compared to the corresponding spectrum obtained in DMSO (695 nm, **Figure 3.8b**). This is due to the differences in the refractive indices of the solvents used. Broad bands in the spectra of **11**-PLL was also observed in water; this is also attributed to the high degree of π - π interaction in this solvent (**Figure 3.9**).

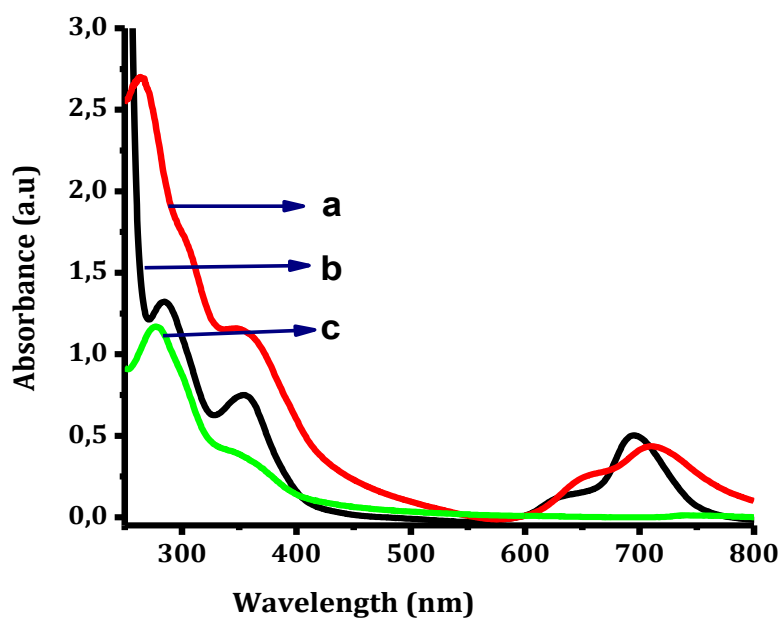


Figure 3.8. : Ground state absorption spectra of 10-FA in water (a), 10-FA in DMSO (b) and folic acid in water

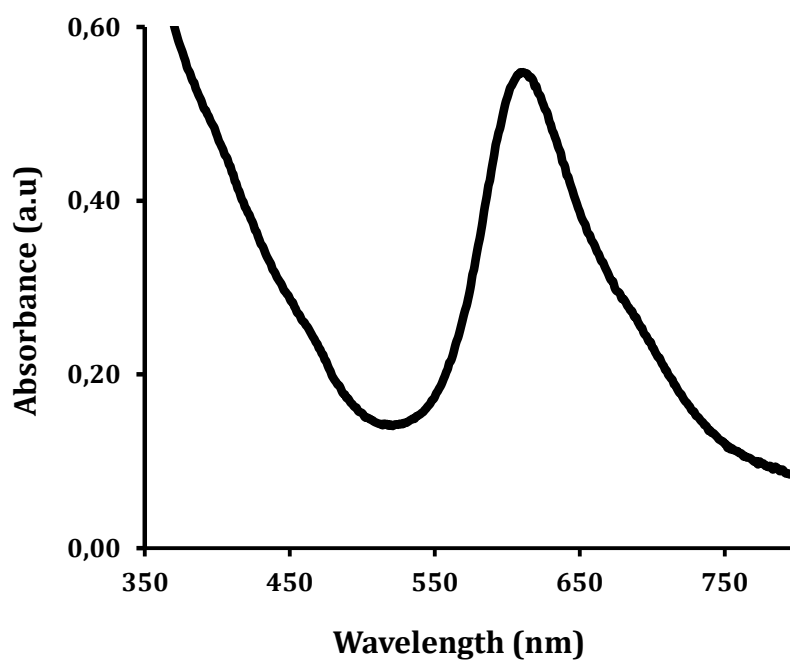


Figure 3.9. Absorption spectrum of 11-polylysine in water as a solvent.

3.3 Phthalocyanine-nanoparticle conjugates

Table 3.2 shows all the Pc nano or micro particle conjugates employed in this work. Complex **7a** was linked to both ZnOMPs and AgNPs, while **7b** and **8** were linked to AgNPs only.

Table 3.2: Summary of nano- or micro-particles conjugated to the reported Pcs. Sizes were determined by SEM or TEM.

Complex	NPs/ MPs (particle size)	Conjugate (particle size)
7a	ZnO (0.4 - 1.8 μm)	7a-ZnOMPs (0.5 - 1.8 μm)
	Ag (2-7 nm)	7a-AgNPs (5-30 nm)
7b	Ag (2- 7 nm)	7b-AgNPs (5-30 nm)
8	Ag (2- 8 nm)	8-AgNPs (6 – 27 nm)
10	Ag (2- 6 nm)	10-AgNPs (3-28 nm)

3.3.1 Complex 7a-ZnOMPs conjugates

The synthesis of ZnOMPs was achieved by using the method reported by Shaheer *et al.* [202]. The particles obtained were microflakes as reported in literature (**Figure 3.10**) [202]. The size of these microparticles ranged from 0.4 to 1.8 μm , **Table 3.2**, thus, they are termed microparticles rather than nanoparticles. The SEM image of the prepared microparticles is accompanied by the size distribution, **Figure 3.10**.

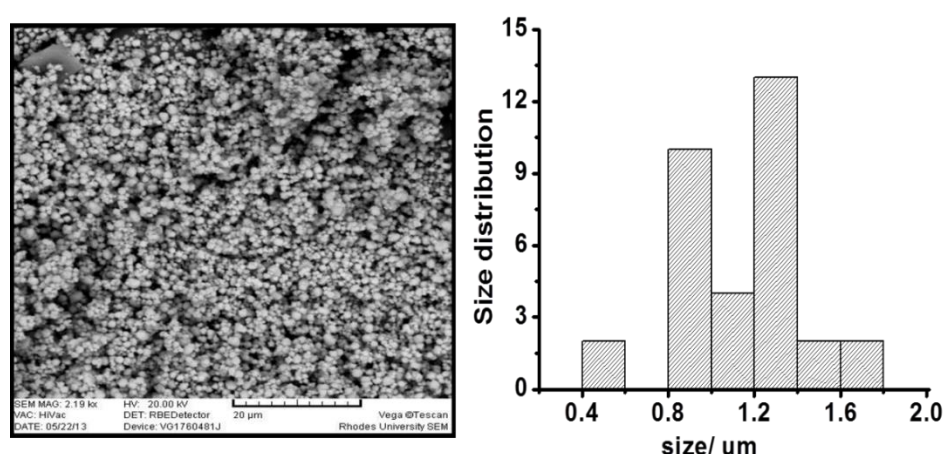
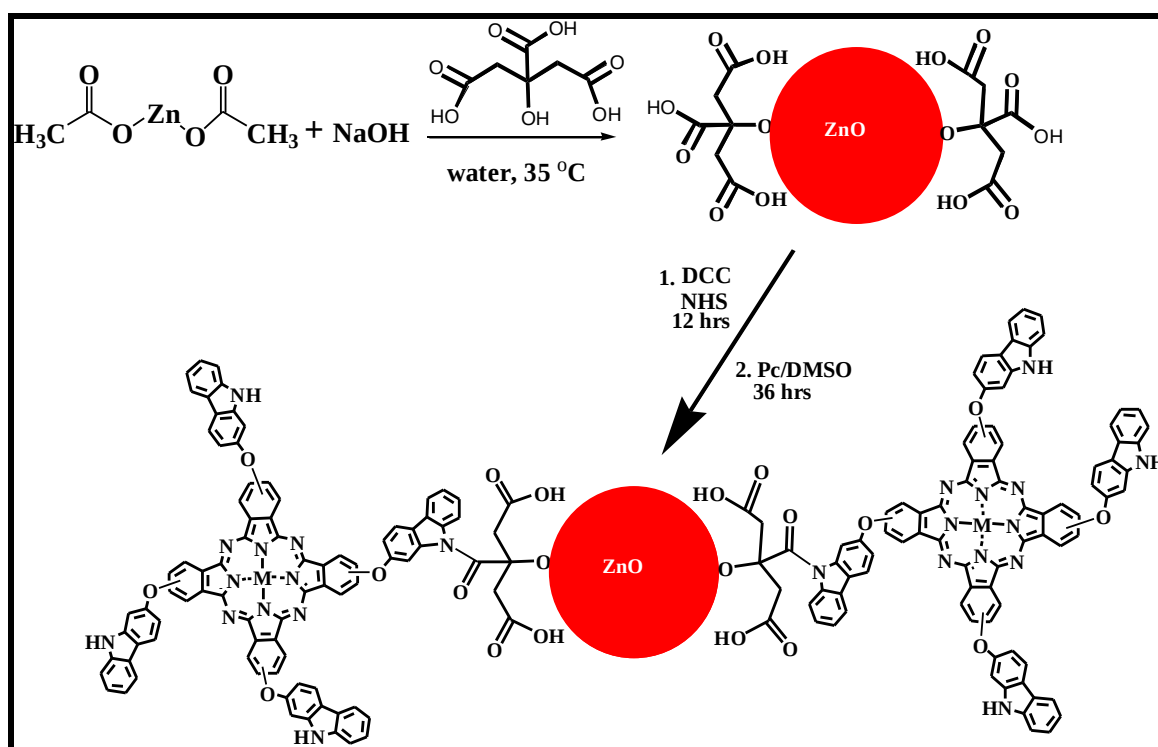


Figure 3.10: SEM image of ZnOMPs and the corresponding particle size distribution.

Conjugation of complex **7a** to ZnOMPs was achieved by activating the carboxylic acid functional group on the ZnOMPs with DCC which is a coupling agent **Scheme 3.6**. It is possible for more than one **7a** to coordinate to ZnOMPs, however, due to size consideration, it is improbable for more than one ZnOMPs to be coordinated to **7a**. The amide bond was formed via a condensation reaction between the activated carboxylic acid group on ZnOMPs and the secondary aromatic amine on **7a**. The formation of the amide bond was confirmed with the IR (**Figure 3.11**) which shows the appearance of a new peak at 1750 cm^{-1} , which is due to the C=O stretching of the amide bond. After conjugating **7a**, there were no significant changes on ZnOMPs particle size (**Table 3.2**).



Scheme 3.6: Synthesis and interaction of ZnOMPs with **7a**. The carboxylic acids of the capping agent are activated to form the amide bond with the secondary amine of **7a**.

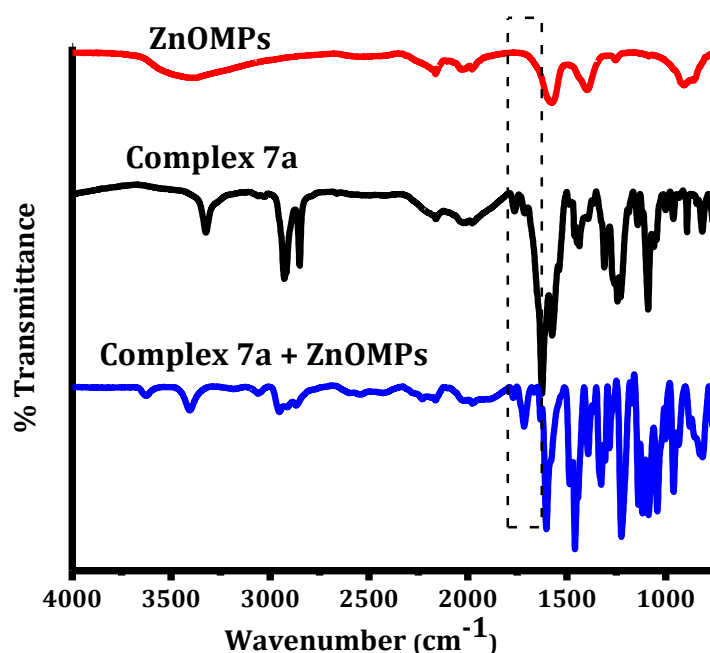


Figure 3.11: IR spectrum of the conjugate, 7a, ZnOMPs and the conjugate of 7a-ZnOMPs.

The purity and crystallinity of the formed ZnOMPs were confirmed by the XRD, Figure 3.12. The sharp, intense peaks in the XRD indicate that ZnOMPs are highly crystalline. The peaks in the XRD pattern were observed at $2\theta = 31.95^\circ, 34.56^\circ, 36.40^\circ, 47.68^\circ, 56.83^\circ, 62.98^\circ, 66.53^\circ, 68.12^\circ, 69.27^\circ$ and 76.97° and were indexed to the (100), (002), (101), (102), (110), (103), (200), (112), (201), and (202) planes of the ZnO crystal, respectively, as referenced by the Joint Committee on Powder Diffraction Standards (JCPDS), card number 01-075-0576. The formation of a pure hexagonal-wurtzite phase was implied by the XRD peaks at the (100), (002) and (101) planes for the ZnO structure. In addition, the narrow peaks imply a larger particle size for the ZnOMPs, confirming the results obtained using SEM. In Figure 3.12, the broad, shallow peak at $2\theta = 20^\circ$ is due to the amorphous nature of the Pc [214]. The sharp peaks in the XRD

pattern of the conjugate are due to ZnOMPs. Thus, the XRD pattern of the conjugate clearly shows the presence of highly crystalline ZnOMPs.

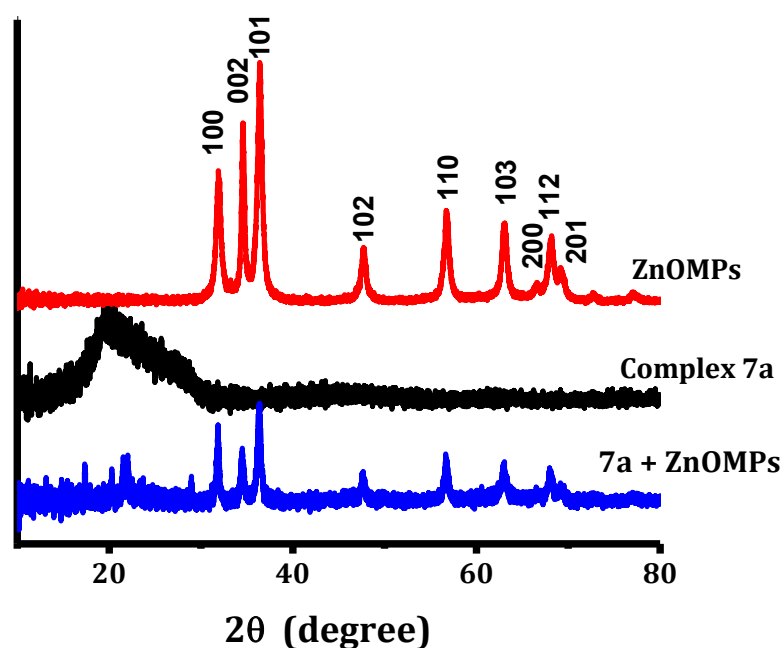


Figure 3.12 XRD pattern of ZnOMPs, complex **7a** and **7a**-ZnOMPs conjugate.

The onset of the absorption for bulk ZnO at room temperature has been reported to be at 370 nm [215]. Citric acid capped ZnOMPs show a distinct absorption peak at ~350 nm (**Figure 3.13**); where the peak is blue shifted when compared to the bulk. The blue shift is due to quantum confinement effect. The tailing of the absorption spectrum is due to poor size dispersity of the microparticles. Upon conjugating complex **7a** to citric acid capped ZnOMPs, the B band was observed to be blue shifted compared to unconjugated **7a**, and the Q band showed no shifting (**Figure 3.13**).

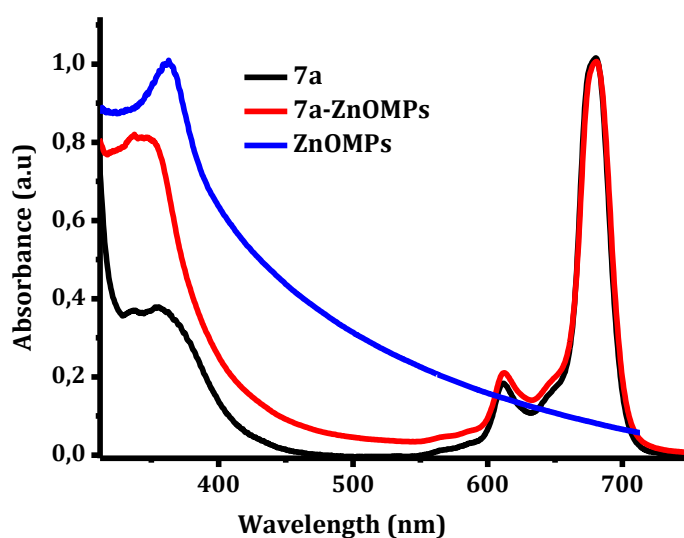
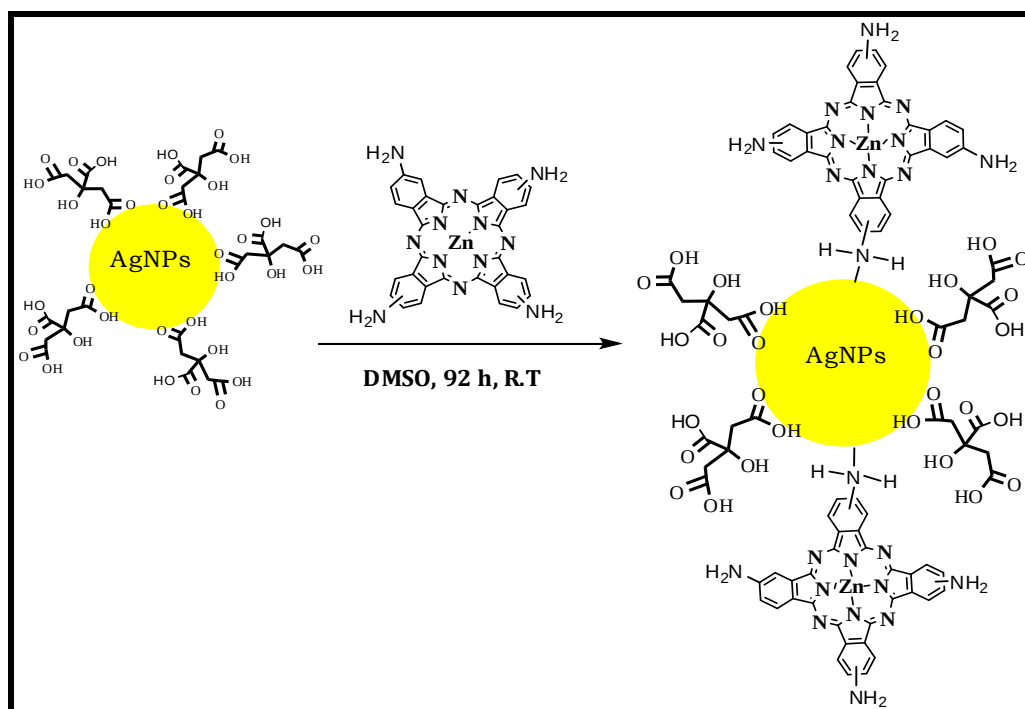


Figure 3.13: UV-Vis spectra of **7a** and when covalently conjugated to ZnOMPs. Spectra were acquired in DMSO as a solvent.

3.3.2 Phthalocyanine-AgNPs conjugates

Citric acid capped silver nanoparticles were synthesized as reported in literature [4], and conjugated to complexes **7a**, **7b**, **8** and **10**. Silver is well known for its high affinity for O and N [216,217]. The interaction between the MPcs and silver nanoparticles is *via* the secondary and primary amine of the carbazole and tetraamino ZnPcs, respectively, **Scheme 3.7**; while with complex **8**, it is proposed that the interaction occurs via the oxygen atom of the ketal ligand since the lone pairs of nitrogen are occupied. Conjugation was achieved by ligand exchange reaction, where some of the citrate capping is replaced by the Pc. It is possible for more than one MPc to be coordinated to one AgNP (**Scheme 3.7**). The reverse, where more than one AgNP coordinates to a Pc, is unlikely due to size consideration. It is also possible for Pcs to

coordinate to each AgNP using more than one of the peripheral substituents, depending on orientation.



Scheme 3.7 : Schematic representation of the interaction between silver nanoparticles and 10.

Fig. 3.14 A and B show TEM images (with corresponding size distribution) of citric acid capped silver nanoparticles before and after conjugation (for **7a** and **7a**-AgNPs as an example). The particles are spherical in shape with sizes ranging from 2 to 7 nm and 5 to 30 nm before and after conjugation, respectively, (**Table 3.2**). There was a significant increase in the size of nanoparticles after the introduction of the Pc. Reports have shown that citric acid as a capping ligand induces electrostatic repulsion between the neighboring nanoparticles [218]. When citric acid (capping agent) is replaced with

MPcs (which are prone to aggregation), nanoparticles coalesce, forming larger particles as depicted by the TEM. A similar increase in size was observed for **7b**, **7b**-AgNPs, **8**, **8**-AgNPs, and **10**, **10**-AgNPs (Table 3.2).

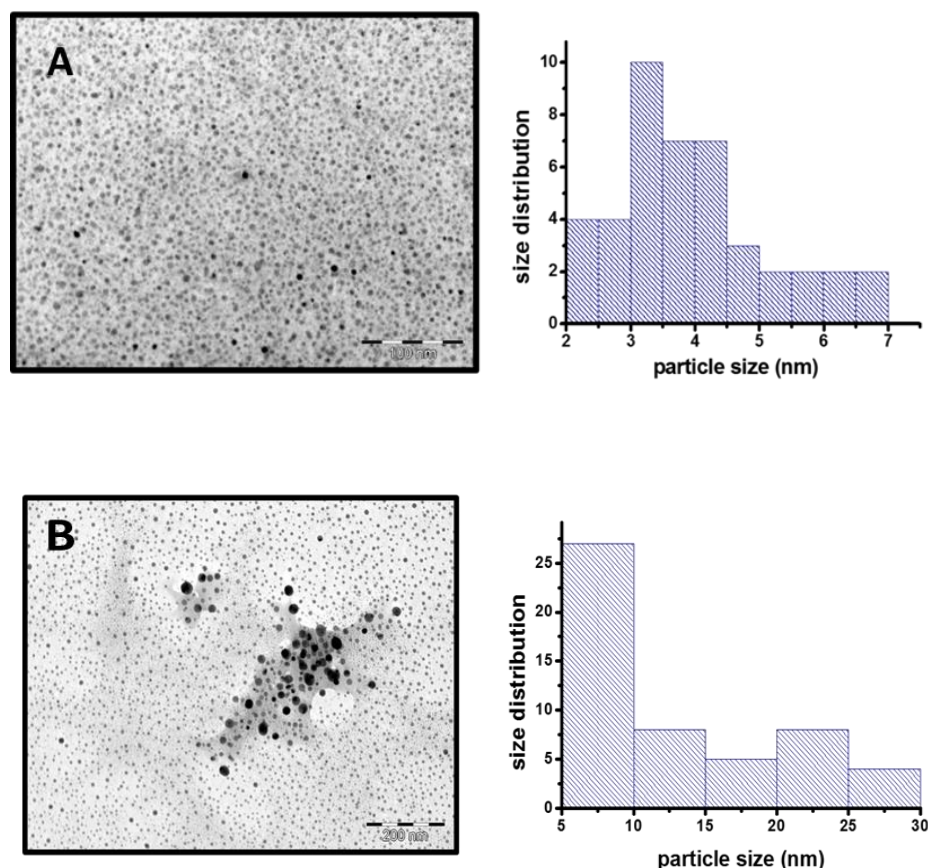


Figure 3.14: TEM images, with the corresponding size distribution of AgNPs (**A**) and **7a**-AgNPs conjugate (**B**) in DMSO as a solvent.

Figure 3.15 shows the UV-Vis spectra of Ag nanoparticles, **7a**, **10** and the conjugates (as examples). An intense, and sharp localized surface plasmonic resonance (LSPR), typical of AgNPs, appeared at ~ 430 nm. In the presence of the Pcs (**7a**, **7b**, **8** or **10**) there was clear red shifting and broadening of the LSPR band, this can be attributed to aggregation of AgNPs upon conjugation. The results are in agreement with the TEM

images in **Figure 3.15**. Aggregation results in red shifting of the LSPR band [218]. Similar to **7a** and **10**, there were no changes in the Q band absorption upon conjugation of the silver NPs to **7b** and **8**.

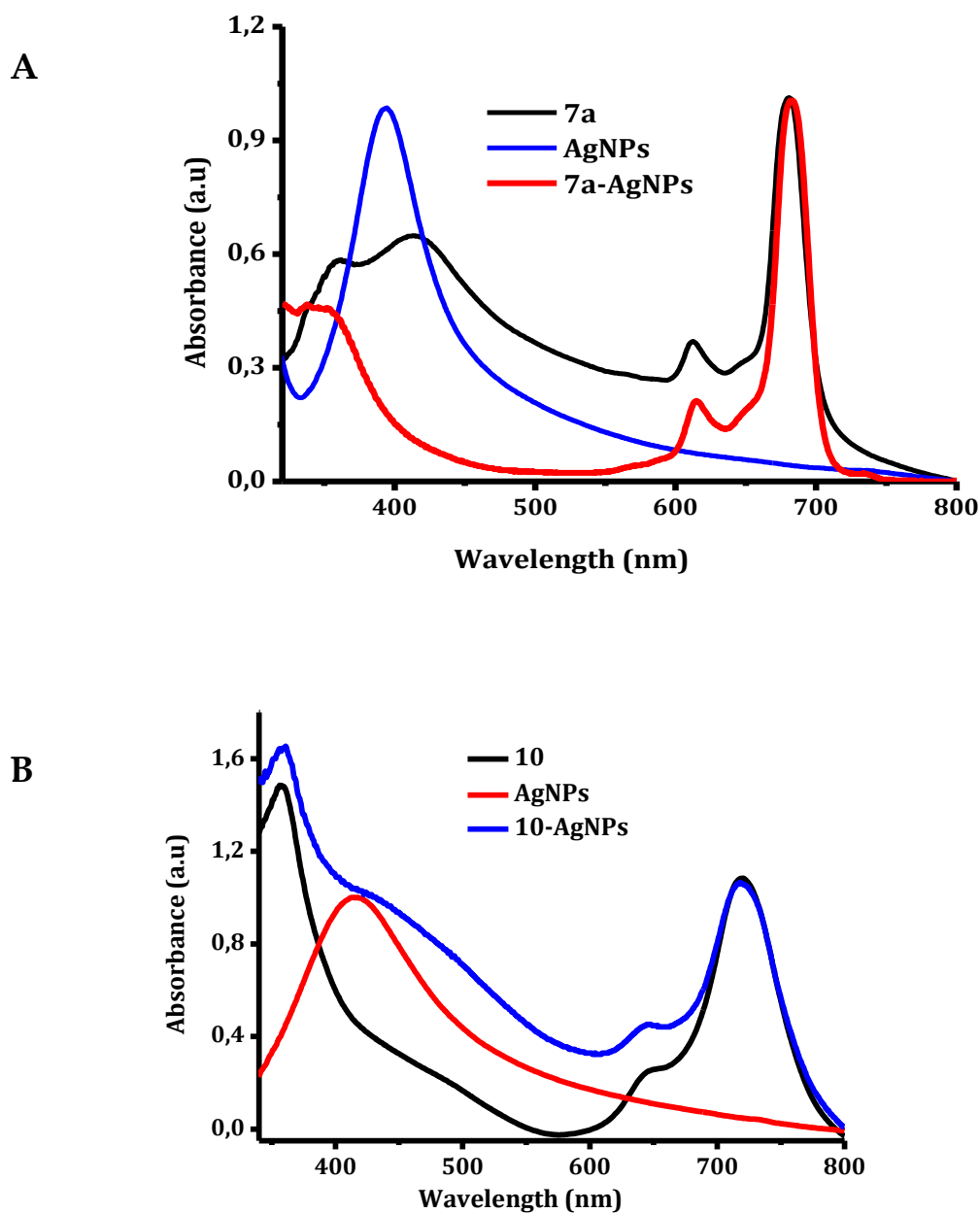


*Figure 3.15: UV-Vis spectra of **7a** and **10** upon conjugating to AgNPs in DMSO as a solvent.*

3.4 Characterization of supports: electrospun fibers and chitosan beads

Table 3.3 lists the type of supports and the immobilized complexes prepared in this study. The characterization of the prepared composites is discussed below. The idea behind electrospinning and the preparation of beads is to obtain an optimum hybrid composite for various applications including waste water disinfection. The prepared supports are insoluble in water, making it easy to recover after use. The insolubility of the individual components of the hybrid composites in water is also an advantage, as this implies that the content of the fiber or beads will not leach out when used in aqueous media.

Table 3.3: Summary of complexes and conjugates immobilized on PS fibers or chitosan beads.

Type of support	Immobilized complex	Abbreviation	Size of supports
PS fibers	-	PS	3-4 μm
PS fibers	AgNPs	AgNPs/PS	3-8 μm
PS fiber	ZnOMPs	ZnOMPs/PS	3-6 μm
PS fibers	7a	7a /PS	3-6 μm
PS fiber	7a -ZnOMPs	7a -ZnOMPs/PS	3-6 μm
PS fiber	7a -AgNPs	7a -AgNPs/PS	3-8 μm
PS fiber	7b	7b /PS	3-6 μm
PS fibers	7b -AgNPs	7b -AgNPs/PS	3-8 μm
Chitosan	-	Chitosan	452 μm
Chitosan beads	10	10 / chitosan	452 μm
Chitosan beads	10 -AgNPs	10 -AgNPs/ chitosan	452 μm

PS= polystyrene

3.4.1 Scanning electron microscopy (SEM).

Functionalized electrospun PS fibers (**7a**/PS, ZnOMPs/PS, **7a**-ZnOMPs/PS, **7a**-AgNPs/PS, AgNPs/PS, **7b**/PS, **7a**-AgNPs/PS and PS) were characterized by scanning electron microscopy. The electrospun fibers were cylindrical and unbranched with relatively smooth surfaces, **Figure 3.16**. Electrospun unfunctionalized polystyrene gave diameter sizes in a range of 3–4 μm (**Figure 3.16**, **Table 3.3**). The diameters for **7a**/PS, ZnOMPs/PS, **7a**-ZnOMPs/PS, **7a**-AgNPs/PS, AgNPs/PS, **7b** and **7b**-AgNPs ranged between 3 to 8 μm , **Table 3.3**. Functionalizing fibers with

ZnOMPs produced nanofibers which showed “white spots” embedded within (Figure. 3.16D). The spots were proven to be ZnO microparticles by EDX (Figure 3.16F). The EDX (Figure. 3.16E and F) shows the elemental composition of these spots, and clearly outlines the high concentration of Zn and O (Figure. 3.16F) as compared to neighboring areas (i.e. areas without white spots, Figure 3.16E).

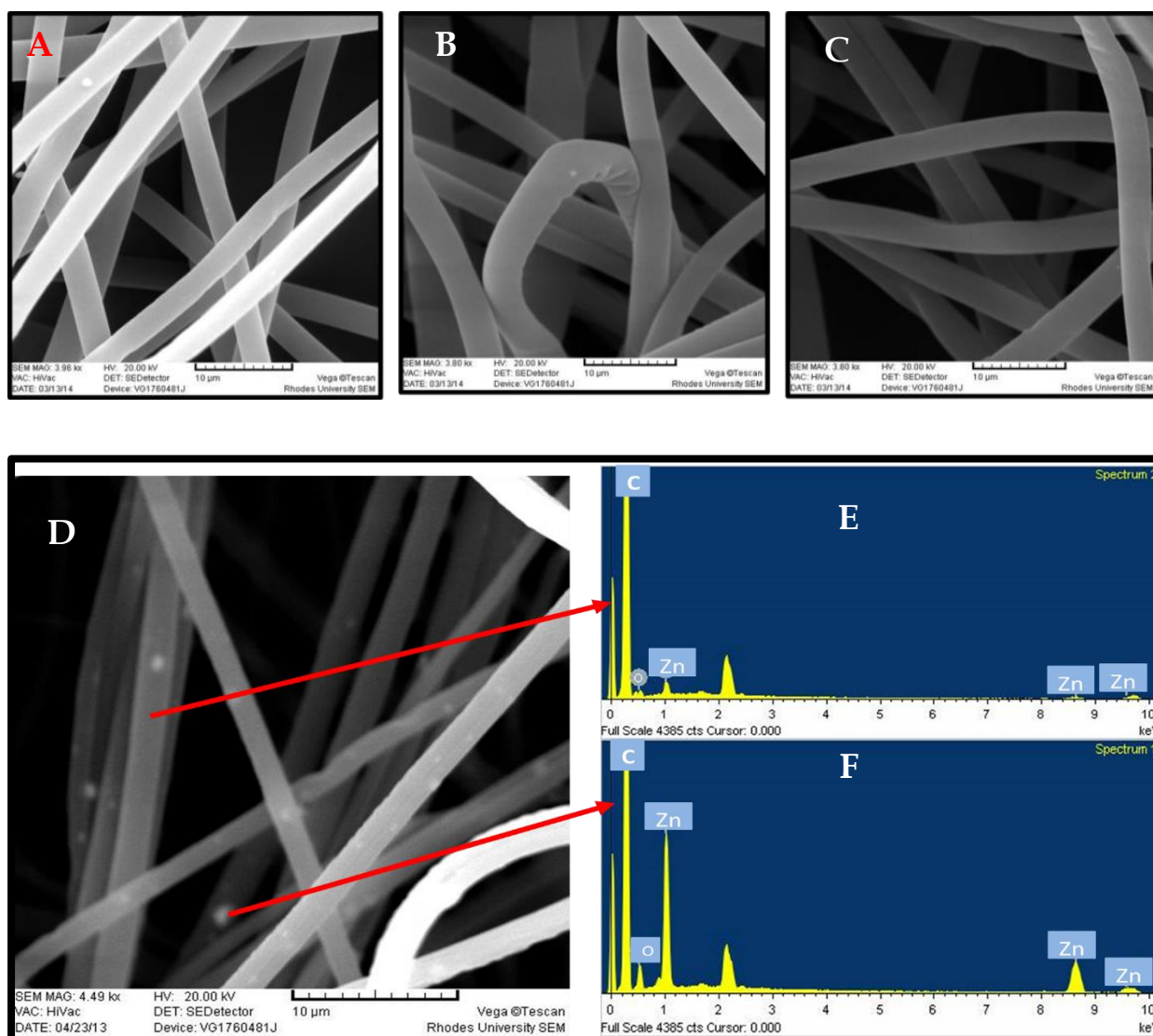


Figure 3.16: SEM micrographs of PS (A), 7a/PS (B), 7a-ZnOMPs/PS (C) and ZnOMPs/PS (D). EDX plots corresponding to the 7a-ZnOMPs/PS fiber with (E) and without (F) ZnOMPs.

The other support used in this study is chitosan microbeads. Complex **10** or **10** conjugated to AgNPs (as xamples) were immobilized onto chitosan beads. The SEM image, **Figure 3.17A** of the dried **10**-AgNPs/chitosan beads was used to determine the size and shape of the prepared beads. The average size of the beads was found to be 452 μm , **Table 3.3**. EDX (**Figure. 3.17B**) was used to confirm the elemental composition

of the prepared beads and showed the presence of residual sodium; this is from the 2 M NaOH that was used to precipitate out chitosan beads, Zn peak of the Pc appears in this region as well, which justifies the intensity of this peak. EDX does however confirm the presence of silver nanoparticles.

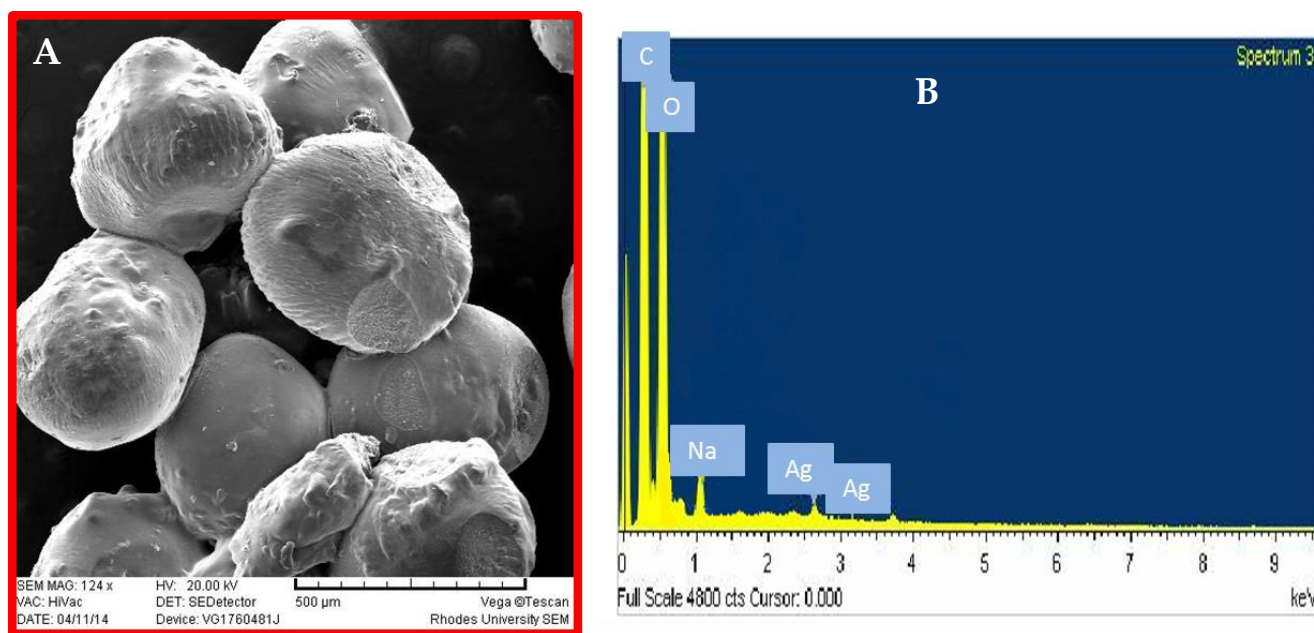


Figure 3.17 SEM images of **10-AgNPc/chitosan** (A) after drying at 60 °C with the corresponding EDX (B).

3.4.2 Fluorescence microscopy

The ability of the nanofibers to emit light upon functionalization was examined with the fluorescence microscopy. ZnOMPs/PS (**Figure 3.18a**) showed fluorescent spots, and the image correlates to that obtained from the SEM, further confirming that the fluorescent spots, or the whitish spots observed on the SEM are ZnOMPs embedded within the fiber. However, fibers immobilized with AgNPs alone did not show substantial fluorescence (figure not shown). In the presence of complex **7a**, the fiber is

observed to have remarkable emission upon excitation (**Figure 3.18b**). Similar fluorescence was observed with excitation of **7a**-ZnOMPs/PS (**Figure 3.18c**), although with weaker emission. Similar fluorescence behaviour of the fiber was observed when **7a** was conjugated to AgNPs, and with **7b** and **7b**-AgNPs. However, fluorescence was not observed upon exciting the microbeads functionalized with **10**-AgNPs or unconjugated complex **10** since NH_2 quenches fluorescence [219]. Unfunctionalized PS fibers or chitosan beads did not show fluorescence either.



Figure 3.18: Fluorescence micrographs of ZnOMPs/PS (**a**), **7a**/PS (**b**) and **7a**-ZnOMPs/PS (**c**).

3.4.3 FTIR spectra

FTIR was used to examine the chemical and structural changes of chitosan upon functionalization. **Figure 3.19** shows the FTIR spectrum of pristine chitosan, **10** and the functionalized chitosan beads. Characteristic chitosan absorption bands appear at 3334 (O-H and N-H stretching vibrations), 2876 (C-H stretching vibrations), 1625 (N-H bending vibrations), and 1056 cm^{-1} (C-O-C stretching vibrations), **Figure 3.19A**, as observed in literature [220,221]. The IR spectrum of **10**/chitosan (**Figure 3.19C**) shows a significant shift of the C=C stretch for **10** that appeared at 1608 cm^{-1} to 1739 cm^{-1} . The

spectral shift is attributed to changes in the chemical environment of **10** upon functionalization into chitosan. Similar changes were observed in the spectrum of **10**-AgNPs immobilized onto chitosan. IR spectra of the fiber is not presented since reports have shown that no changes are observed for the functional groups of PS upon immobilizing different complexes with the physisorption method [152,153].

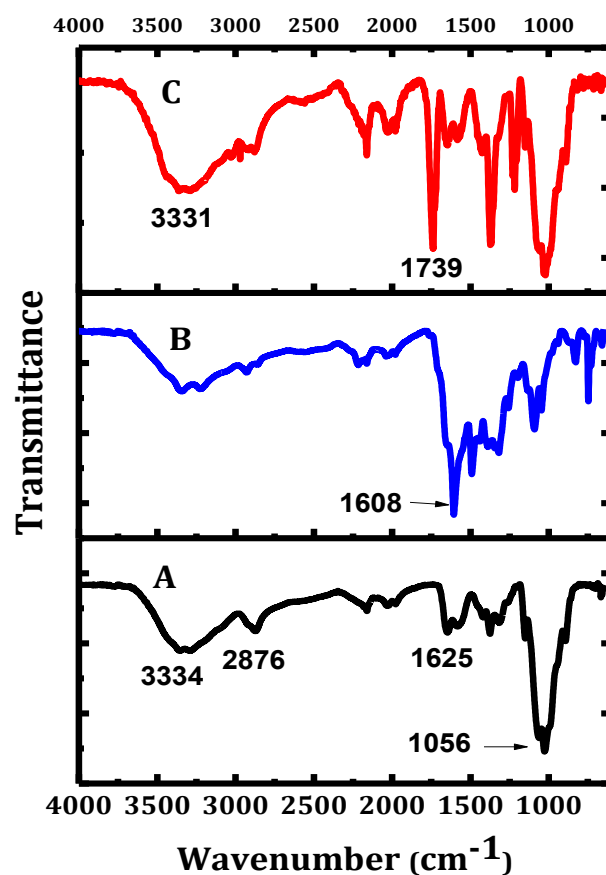


Figure 3.19: FTIR spectra of chitosan (A), 10 (B) and 10/chitosan (C) beads dried at 60 °C

3.4.4 TGA/DTA analysis.

The effect of heat on the prepared supports was examined by thermal analysis. Changes in weight with an increase in temperatures were monitored by

thermogravimetric analysis (TGA). **Figure 3.20A** shows that the modified fibers are slightly more stable than unfunctionalized polystyrene. Differential thermogravimetric analysis (DTA) was acquired simultaneously with the thermogravimetric analysis; therefore, the results obtained are comparable. DTA gives the apparent degradation temperature, the derivative weight losses of the composites and polystyrene alone are shown in **Figure 3.20B**. The DTA curve of ZnOMPs/PS is clearly shifted to higher temperatures, indicating the greater thermal stability of the composite. The thermal stabilities of **7a**/PS and **7a**-ZnOMPs/PS were found to be similar, but slightly improved when compared to pristine polystyrene, as judged by the small shift to higher temperatures.

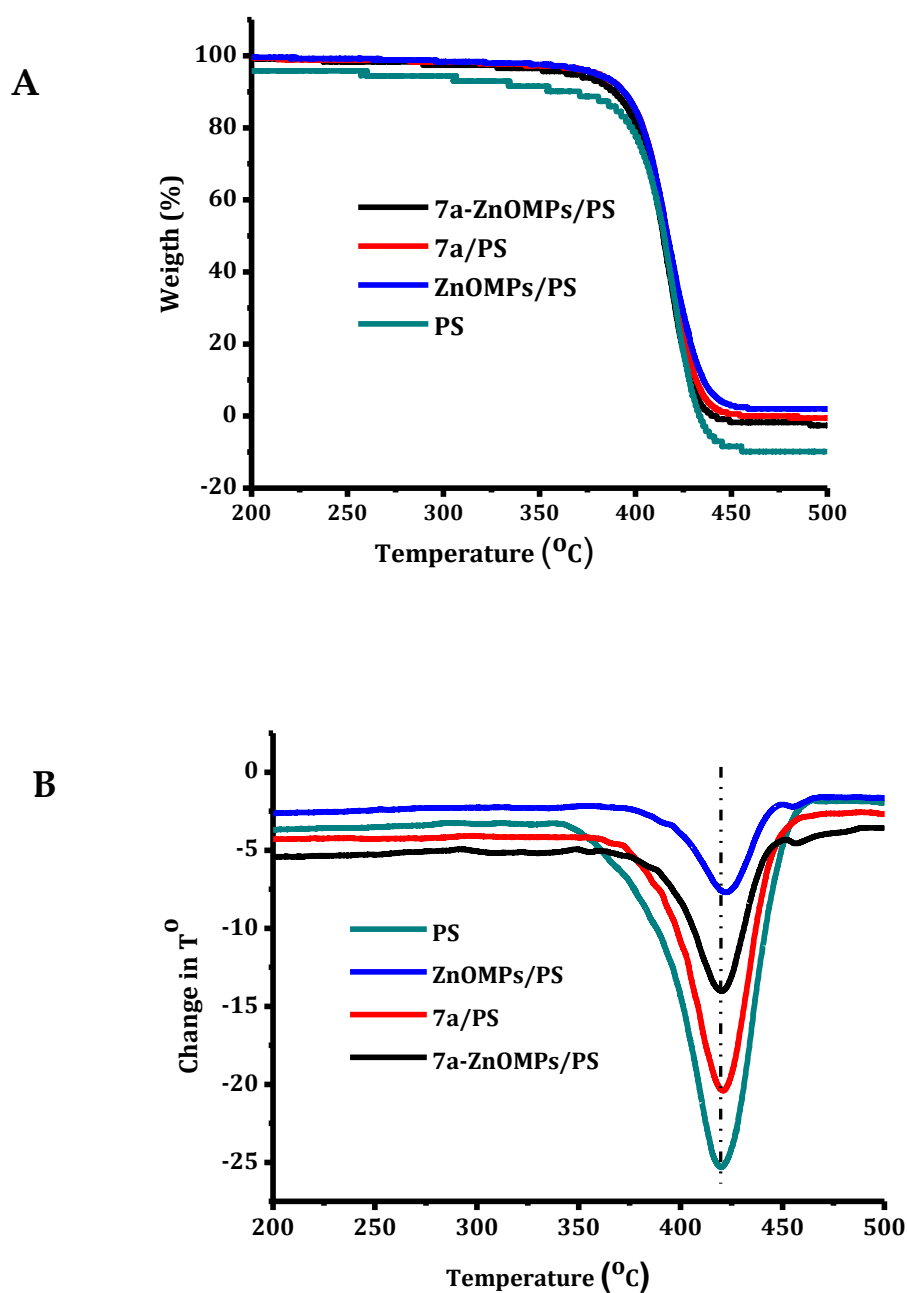


Figure 3.20: TGA (A) and DTA (B) thermograms of PS and PS functionalized nanofibers.

The thermograms (**Figure 3.21**) of unfunctionalized chitosan showed a sharp weight loss at 268 °C. The temperature for this weight loss increased to 286 and 290 °C for

10/chitosan and **10**-AgNPs/chitosan, respectively, suggesting improved stability of the beads upon functionalizing.

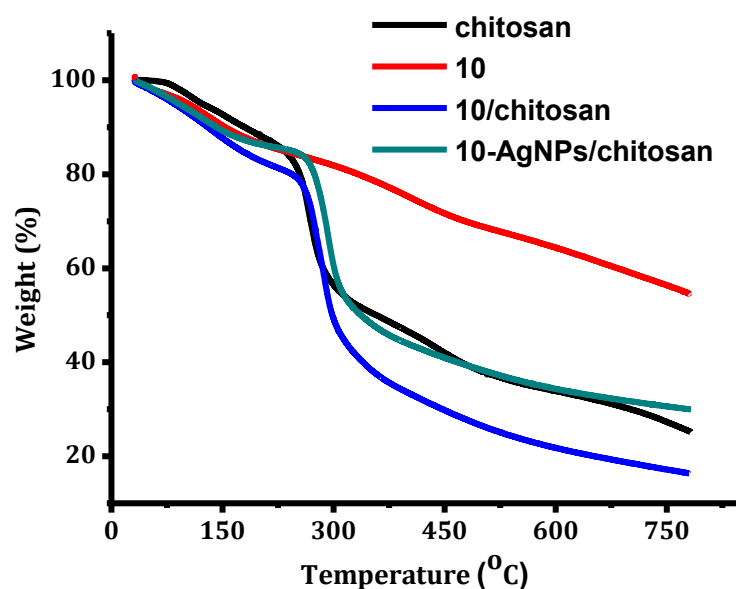


Figure 3.21: Thermograms of **10**, chitosan beads and chitosan beads functionalized with **10** and **10**-AgNPs.

3.4.5 Ground state absorption spectra

The fibers of **7a**/PS, ZnOMPs/PS and **7a**-ZnOMPs/PS were not soluble in water, therefore, were dissolved in toluene with the aim of examining the integrity of the components after spinning. UV-Vis spectra of the fiber composites (in toluene) are shown in **Figure 3.22**, with a characteristic Q band at 683 nm. The integrity of the components (complex **7a** or **7a**-ZnOMPs) in the electrospun fiber is not compromised upon electrospinning, since there were no changes in the Q band maxima. Similar

spectra were obtained when **7b**/PS, **7b**-AgNPs/PS, **7a**-AgNPs/PS fibers were dissolved in toluene. Microbeads were insoluble in most solvents, therefore, the integrity of the components of the beads was not examined with the UV-Vis.

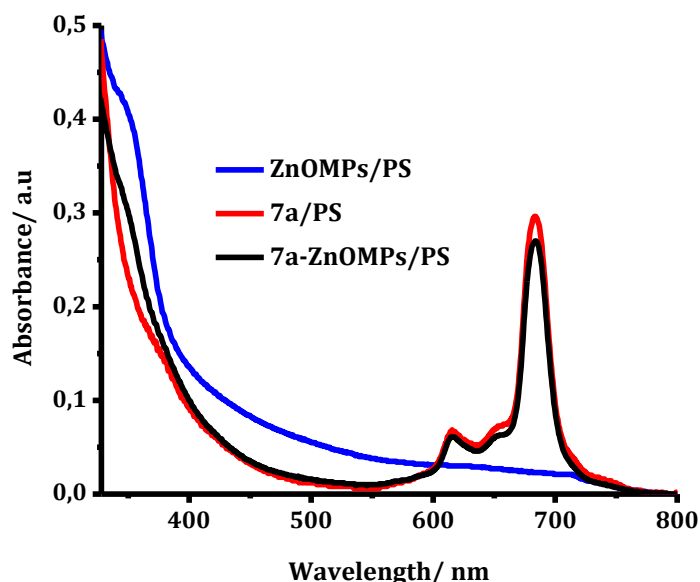


Figure 3.22: Absorption spectra of **7a**/PS, **7a**-ZnOMPs/PS and ZnOMPs/PS dissolved in toluene.

3.4.5 X-Ray powder diffraction patterns

The XRD pattern of the electrospun fibers is shown in **Figure 3.23**. The XRD pattern of PS shows a broad peak at $2\theta = 20^\circ$, where phthalocyanines also show a peak [214], **Figure 3.23**. This broad peak for PS is close to the 002 reflection peak for carbon [214]. The broadness of the peak is an indication of the amorphous nature of polystyrene electrospun fibers. Functionalization of polystyrene gave diffraction patterns that are mainly characteristic of polystyrene; there were no significant changes in the diffraction pattern of polystyrene upon functionalization (**Figure 3.23**).

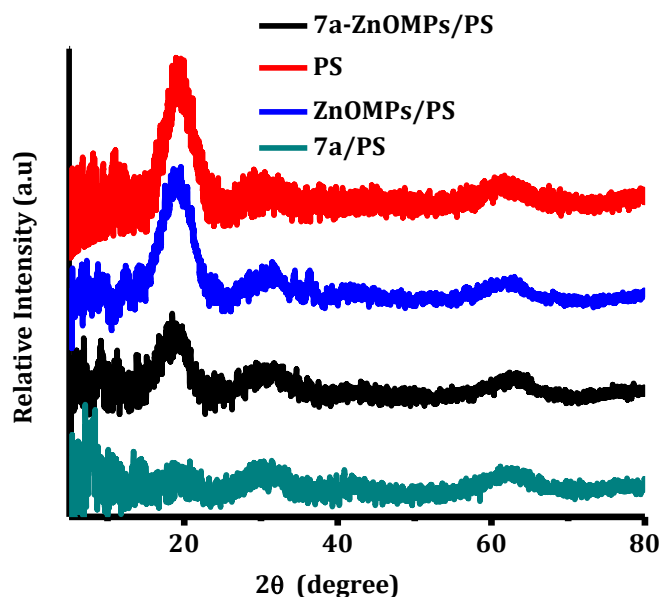


Figure 3.23: XRD pattern of PS, ZnOMPs/PS, 7a/PS and 7a-ZnOMPs/PS.

Figure 3.24 shows the XRD profiles of pristine chitosan, chitosan functionalized with **10** and chitosan functionalized with **10**-AgNPs. As can be seen from this figure, the unfunctionalized chitosan and chitosan functionalized with **10** or **10**-AgNPs showed a peak at $2\theta = 19.9$; the reflection peak matches with the literature value [220]. These results suggest that chitosan's crystallinity was not compromised by immobilizing the photocatalyst. The highlighted additional peaks at $2\theta = 38.04, 44.56, 67.61$ and 77.60 were indexed to 111, 200, 220 and 311 planes of the Ag crystal, respectively, as referenced by the JCPDS card number 00-001-1013. These peaks are only noticeable in **10**-AgNPs/chitosan beads.

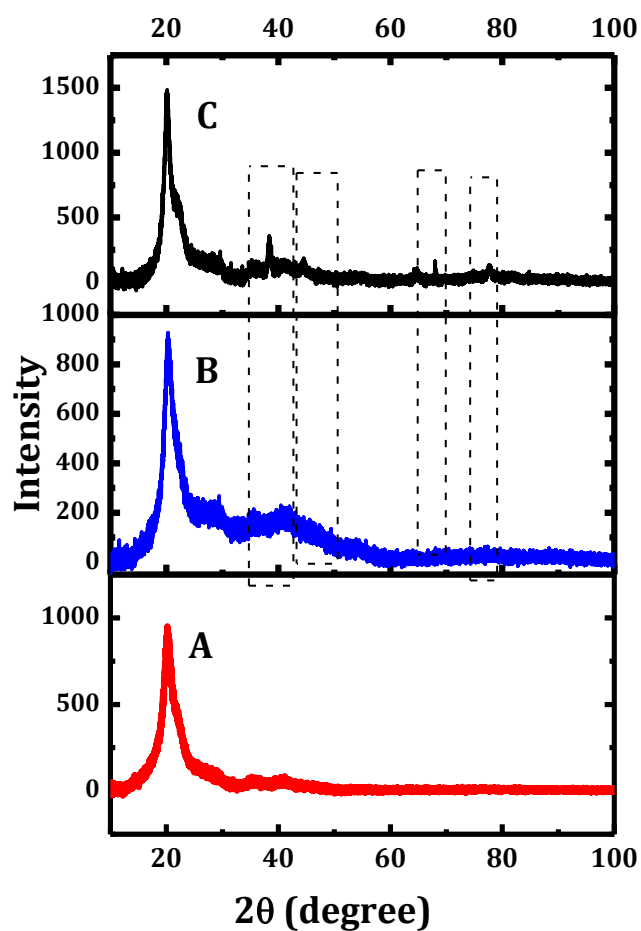


Figure 3.24: XRD diffraction patterns of pristine chitosan (A), 10/chitosan (B) and 10-AgNPs/chitosan (C) beads.

CHAPTER 4

PHOTOPHYSICOCHEMICAL PROPERTIES

The chapter discusses the photophysical and photochemical behaviour of the novel phthalocyanines when conjugated to nanoparticles, targeting moieties and when immobilized onto electrospun fibers and microbeads.

4.1 Photophysical and photochemical behaviour of MPcs

4.1.1 Fluorescence spectra, quantum yields and lifetimes of MPcs

Fluorescence excitation and emission spectra of complexes **7a**, **7b**, **8** and **9** are shown in **Figure 4.1**. The fluorescence emission spectra were mirror images of the excitation spectra; this is typical of non-aggregated phthalocyanine complexes in solution. The proximity of the Q band maxima of the absorption and excitation spectra confirms that the molecule absorbing is the same as the one that is emitting. The prepared complexes (**7a**, **7b** and **9**) gave Stoke's shifts ranging from 8 to 18 nm, which is normal for Pc derivatives. The excitation spectrum of **8** is slightly red shifted from the absorption spectrum (**Figure 4.1C**); this could be due to the change in geometry upon excitation. A single peak is observed in the emission spectra (**Figure 4.1C**). Metal free Pcs are known to fluoresce with only one main peak in non-aqueous media, and this peak has been assigned to the 0-0 transition of the fluorescence process [222].

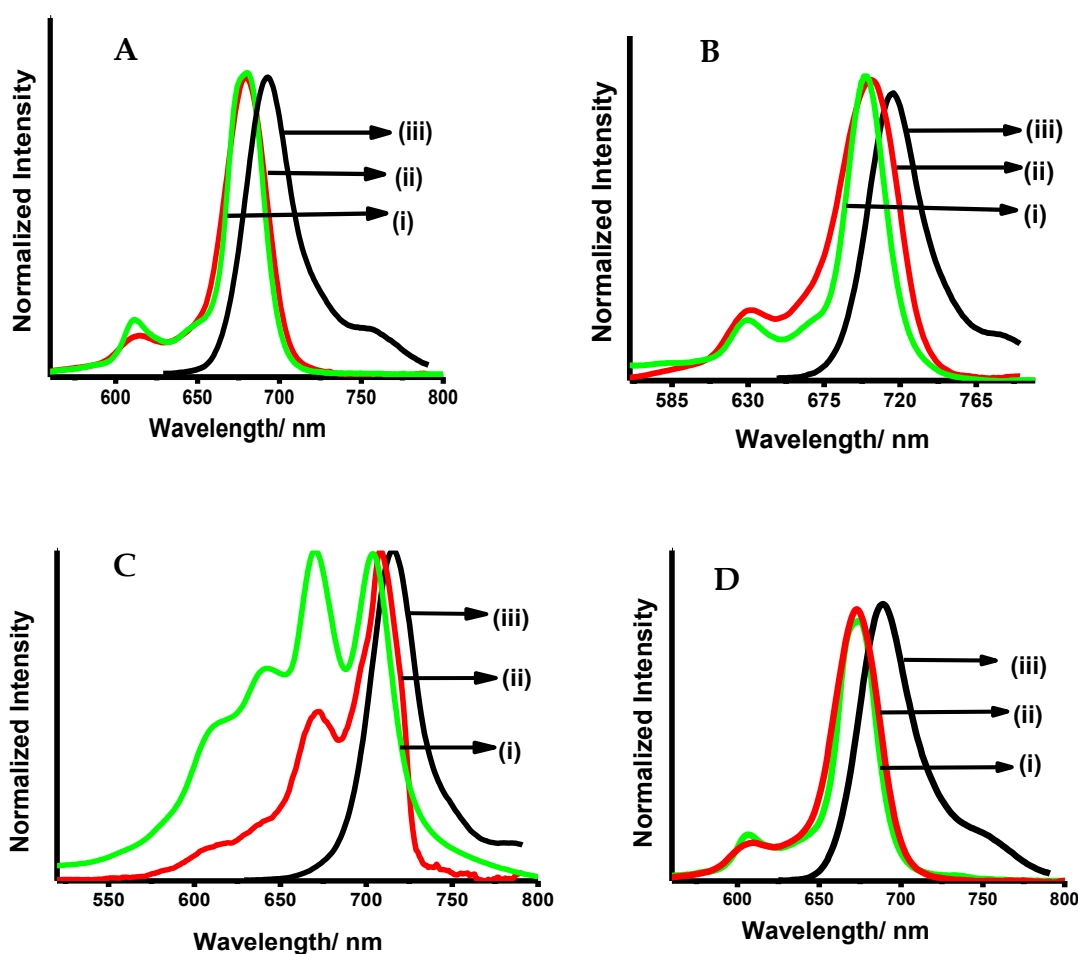


Figure 4.1: Absorption (i), excitation (ii) and emission (iii) spectra of complex **7a** (A), **7b** (B), **8** (C) and **9** (D) in DMSO as a solvent.

The fluorescence quantum yields of the prepared complexes were found to be 0.10, 0.13, 0.21 and 0.095 for complexes **7a**, **7b**, **8** and **9**, respectively, **Table 4.1**. For the Zn based Pcs (**7a**, **7b** and **9**), the quantum yields were lower than that of the unsubstituted ZnPc in DMSO ($\phi_F = 0.20$ [82]) suggesting quenching of fluorescence by the substituents. The free base phthalocyanine (**8**) gave a higher fluorescence quantum yield compared to **7a**, **7b** and **9**, this is due to the heavy atom effect of Zn which encourages the intersystem crossing, therefore, reducing fluorescence in Zn based Pcs. The ϕ_F obtained for complex **9** is lower compared to other Zn based phthalocyanines.

This means that the benzothiazole substituent, in particular, quenches the fluorescence of the parent ZnPc. Fluorescence lifetime refers to the average time a molecule stays in the excited state before emitting a photon. Factors such as internal conversion and intersystem crossing are known to shorten fluorescence lifetime of a fluorophore, which indirectly reduces the fluorescence quantum yield. Using the TCSPC, **Figure 4.2**, the fluorescence lifetimes of **7a**, **7b**, **8** and **9** were found to be 2.83, 2.50, 3.48 and 2.80 ns, respectively (**Table 4.1**, **Figure 4.2**). The lifetimes were within the range expected for most Pcs [82]. All complexes showed mono-exponential decay curves, suggesting that the complexes were pure.

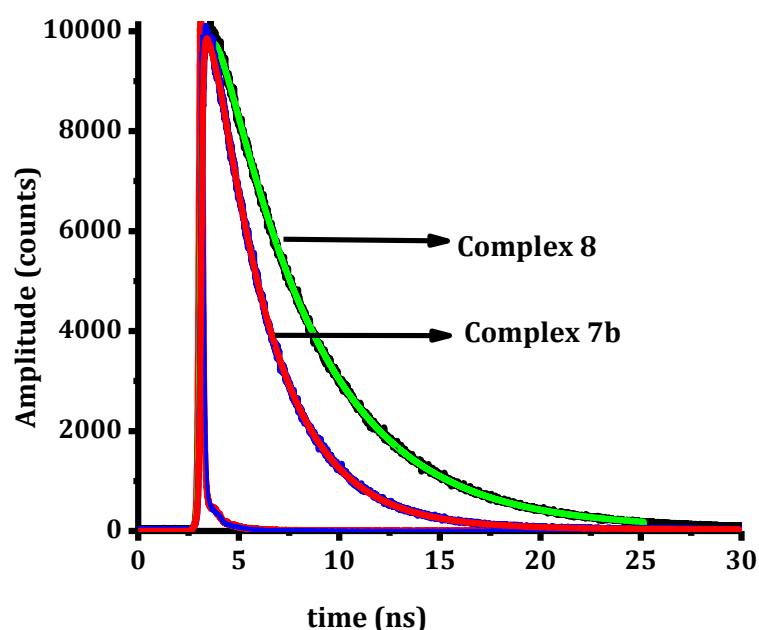


Figure 4.2: Fluorescence decay curves of complex 8 and 7b in DMSO.

4.1.2 Triplet quantum yields and lifetimes of MPcs

The triplet quantum yield (ϕ_T) represents the fraction of molecules that undergo intersystem crossing to the metastable triplet excited state. A high triplet quantum yield with a corresponding low fluorescence quantum yield suggests a more efficient intersystem crossing, which is desirable for a compound that might be used as a photosensitizer. The ability of a molecule to be long-lived and populate the triplet state is of great importance since this has a direct bearing on the singlet oxygen production. **Figure 4.3** shows the triplet decay curve of complex **7b** (as an example) along with its transient curve. The triplet absorption of complex **7a** and **7b** was centred at 540 nm, while those of complex **8** and **9** were at 490 nm. De-oxygenation of solutions is important in accurate determination of the sensitizer's triplet state parameters, since the triplet state of Pcs is known to be quenched by oxygen [223], therefore, prior to experiments, samples were de-gassed by bubbling with argon for 20 min.

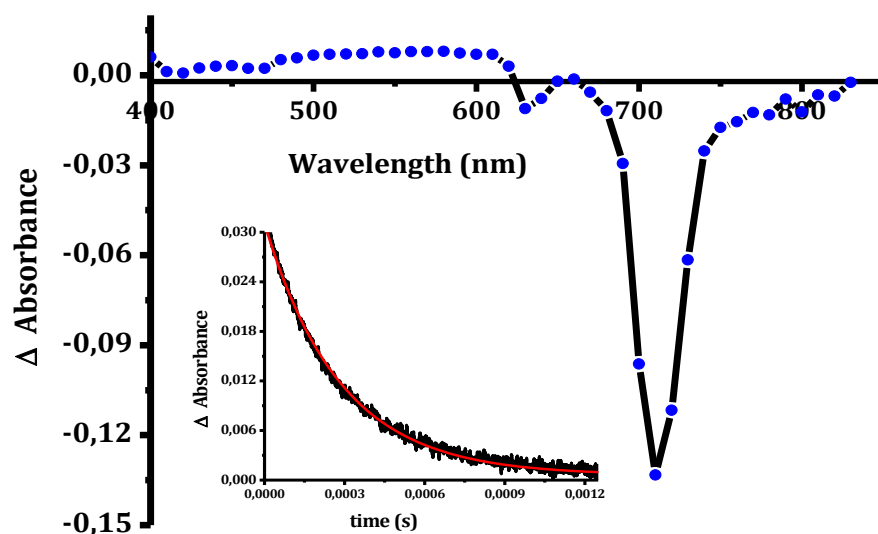


Figure 4.3: Transient curve of complex **7b**, along with **7b** triplet decay curve (Excitation = 540 nm).

The triplet quantum yields of the complexes were found to be 0.69, 0.75, 0.59 and 0.63 for **7a**, **7b**, **8** and **9**, respectively, **Table 4.1**. Complex **9** which has a lower ϕ_F was expected to have a higher ϕ_T , but this is not the case (**Table 4.1**). Non-radiative internal conversion processes (ϕ_{IC}) were found to be much more dominant for complex **9** compared to other complexes, consequently, resulting in a lower triplet quantum yield. High ϕ_T values are usually accompanied by short triplet lifetimes. Even though complex **9** showed a lower triplet quantum yield compared to other Zn based Pcs, the triplet lifetime of **9** was disproportionately long, at 1740 μs (**Table 4.1**). Such long lifetimes are only known for MPcs containing small central metals such as Al or Mg [224]. The long triplet lifetime for **9** may be attributed to the well-known enhancement

of triplet lifetimes by benzothiazole derivatives [225]. This lifetime (1740 μ s) is the longest ever reported for a ZnPc derivative. This shows that the combination of a benzothiazole ring system together with a ZnPc makes for a good photosensitizer.

4.1.3 Singlet oxygen quantum yields of MPcs

The singlet oxygen quantum yield is a measure of the ability of a sensitizer to produce singlet oxygen. Amongst other factors, the efficiency of singlet oxygen generation depends on the energy, quantum yield and lifetime of the triplet state. Singlet oxygen quantum yields were studied using the chemical method discussed in the experimental section. **Figure 4.4** shows the spectral changes observed during the photolysis of complex **9** (as an example) in the presence of DPBF. The disappearance of DPBF was monitored using UV-Vis spectral changes. There were no changes in the Q band intensities during the study, confirming that the complexes do not degrade during the singlet oxygen quantum yield determinations. The singlet oxygen quantum yields were found to be 0.61, 0.69, 0.48 and 0.49 for compounds **7a**, **7b**, **8** and **9**, respectively, **Table 4.1**. When compared to unsubstituted ZnPc (0.67, [54]), complex **9** (0.49) had a lower value, but it is still considered to be high enough to be useful in photocatalytic applications such as PDT.

S_{Δ} ($=\phi_{\Delta}/\phi_T$) values represent the fraction of excited triplet state molecules quenched by ground state molecular oxygen. S_{Δ} is the efficiency of energy transfer from the triplet state of the complexes to ground state molecular oxygen. The closer the S_{Δ} value

is to unity, the higher the efficiency of energy transfer. Complex **7b** showed better energy transfer when compared to the other phthalocyanines reported in this work. Although **8** has the lowest ϕ_T , the energy transfer between the triplet and the singlet state was reasonably high compared to complex **9**. This means that even though complex **8** is unmetallated, it still exhibits remarkable photosensitizing abilities as reported previously for other H₂Pcs [67,68]. The ϕ_Δ value for **7b** is larger than for **7a**, corresponding to the trends observed in the triplet behaviour studies. Any factor that promotes structural distortion will enhance intersystem crossing to the triplet excited state [225]. Non-peripheral substitution supports molecular distortion, hence, the higher ϕ_Δ value was obtained for **7b** compared to **7a**.

Table 4.1: Photophysics and photochemical behaviour of complexes in DMSO

Complex	ϕ_F	τ_F (ns)	ϕ_T	τ_T (μ s)	ϕ_{IC}	ϕ_Δ	S_Δ
7a	0.10	2.83	0.69	359	0.21	0.61	0.88
7b	0.13	2.50	0.75	309	0.12	0.69	0.92
8	0.21	3.48	0.59	412	0.20	0.48	0.81
9	0.095	2.80	0.63	1740	0.28	0.49	0.78

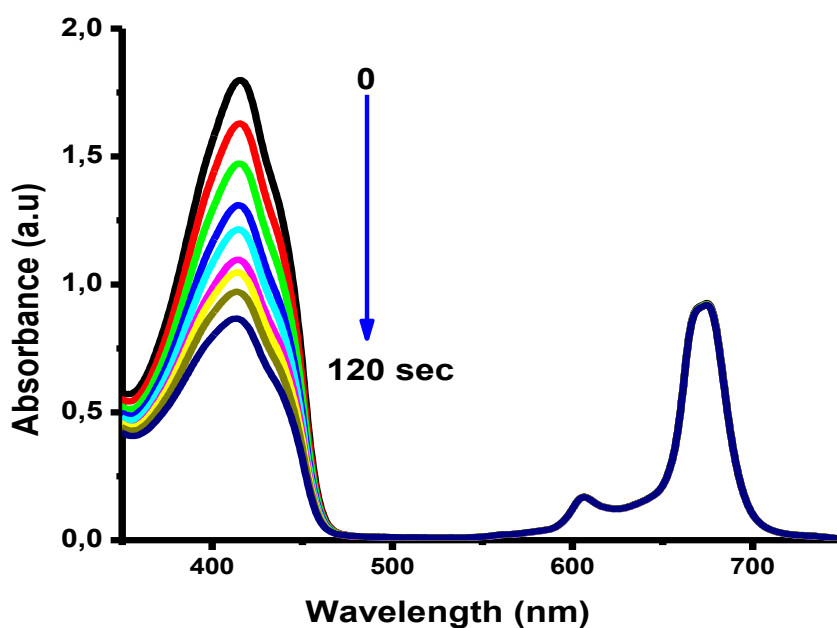


Figure 4.4 A typical spectra for the determination of singlet oxygen quantum yield in DMSO using complex **7a** as an example.

4.2 Photophysicochemical behaviour of 10-folic acid conjugate

4.2.1 Fluorescence spectra, quantum yields (ϕ_F) and lifetimes (τ_F)

Photophysicochemical studies in water are not reported because the high degree of aggregation makes it difficult to obtain significant information in this solvent. The aggregates could not be broken by addition of surfactants. The absorption, fluorescence, and excitation spectra of the conjugate are shown in **Figure 4.5**. The absorption spectrum was similar to the excitation spectrum and both were mirror images of the emission spectrum signifying no changes in geometry upon excitation. It has been reported for porphyrins that fluorescence quantum yields are slightly enhanced by the presence of folic acid [208]. However, for fluorinated

phthalocyanines mixed with folic acid, the fluorescence quantum yields remained the same or became quenched in the presence of FA depending on the central metal [226]. In the case of **10**-FA conjugate, the fluorescence quantum yields increased in the presence of folic acid when either chemically linked or mixed, **Table 4.2**. Due to the quenching effects of the amino group [219,227], low ϕ_A values are known for amino substituted phthalocyanines (as observed for **10**). The increase of ϕ_F values in the presence of FA could be due to a decrease in the quenching effects of the amino groups now that they are engaged with FA.

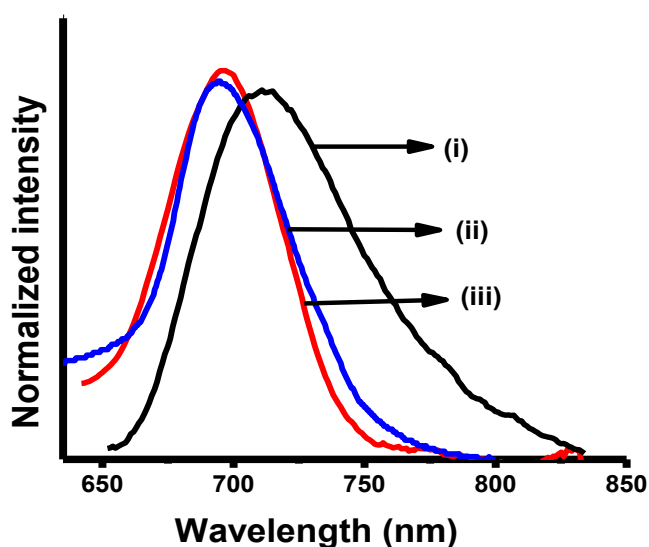


Figure 4.5: Emission (i), absorption (ii) and excitation (iii) spectra of **10**-FA conjugate in DMSO. Excitation wavelength = 640 nm

A single lifetime was obtained for **10** in DMSO. However, a bi-exponential decay curve was observed for **10**-FA conjugate with lifetimes at 2.3 and 1.4 ns, **Table 4.2**. The first, long lifetime is associated with the monomeric non-interacting **10** molecules, while the second lifetime was attributed to the interaction between **10** and folic acid. Such quenched system is short-lived with low abundance. Time-resolved fluorescence

spectroscopy (TRES) was conducted to confirm the origin of the lifetimes observed. **Figure 4.6(i)** shows that the long lived lifetime (2.3 ns) is a result of fluorescence at 729 nm which is also the fluorescence maximum of **10** in DMSO. The emission at ~ 711 nm (1.4 ns) was attributed to the emission of the conjugate, **Figure 4.6 (ii)**.

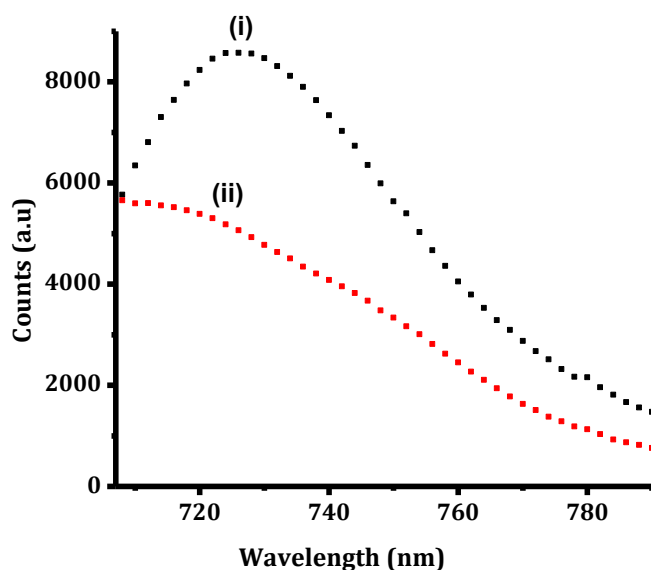


Figure 4.6: Time resolved emission spectra (TRES) of **10-FA** in DMSO. Long lifetime at 2.27 ns (i) and a short lifetime of 1.45 ns (ii)

4.2.2 Triplet quantum yields (Φ_T) and lifetimes

Figure 4.7 shows the triplet decay curve of **10-FA** which obeyed second order kinetics. This is typical of MPc complexes at high concentrations ($< 1 \times 10^{-5}$ M) [228]) due to triplet-triplet recombination. The Φ_T values decreased for **10-FA** compared to **10**, suggesting quenching of the triplet state by linking **10** to FA as opposed to mixing where there was no significant change in the Φ_T value, **Table 4.2**. The close proximity of **10** and FA in the chemically linked **10-FA** conjugates may encourage radiationless

loss of energy through the bonds, hence lowering both the triplet and singlet oxygen quantum yields. The low triplet quantum yield of **10**-FA conjugate was accompanied by an increase in the triplet lifetime. There was also an increase in the lifetime of **10** mixed with FA (**10**+FA) compared to **10** alone, probably due to shielding of **10** by FA.

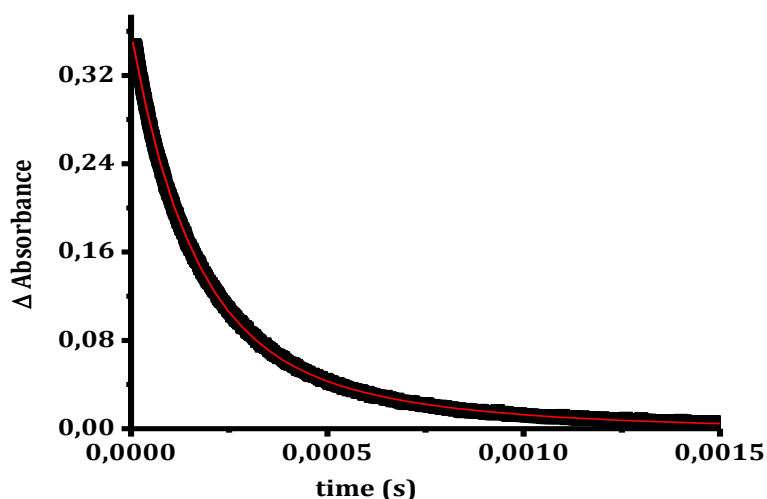


*Figure 4.7: Bi-exponential triplet decay curve of **10**-FA in DMSO.*

4.2.3 Singlet oxygen quantum yields

The amount of singlet oxygen produced by the conjugate was determined by monitoring the disappearance of DPBF spectroscopically over a period of time. There was no change in the Q-band intensity during the ϕ_{Δ} determinations, **Figure 4.8**. Quantum yields of singlet oxygen generation for **10**-FA were lower compared to unconjugated **10** in DMSO, suggesting quenching, **Table 4.2**. For the mixed **10**+FA, there was an increase in the ϕ_{Δ} value. The values of ϕ_{Δ} and ϕ_T were the same for the

conjugate, confirming efficient energy transfer between the triplet state of **10**-FA and triplet state oxygen ($^3\text{O}_2$), Table 4.2.

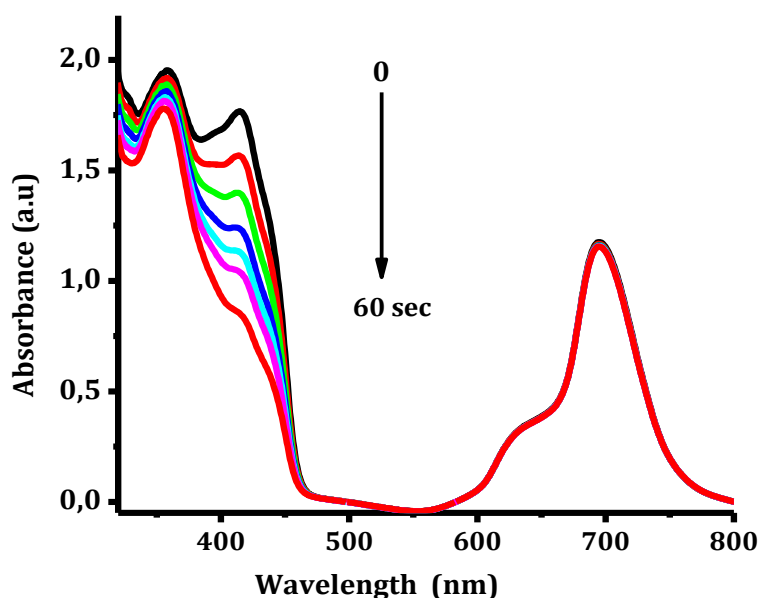


Figure 4.8: A typical spectrum for the determination of singlet oxygen quantum yield. The spectra shows the degradation of DPBF in the presence on **10**-FA.

Table 4.2. Photophysicochemical parameters of **10** and **10**-FA in DMSO

Compound	λ_{max}	ϕ_{Δ}	ϕ_{T}	ϕ_{F}	$\tau_{\text{T}}(\mu\text{s})$	$\tau_{\text{F}}/\text{ns} \pm 0.3$	Amplitudes for τ_{F} values
10	715	0.31	0.50	0.015	99	2.6	1.0
10 -FA conjugate	695	0.21	0.21	0.037	150	2.3	0.9
						1.4	0.1
10 +FA mixed	706	0.39	0.48	0.053	120	2.3	0.7
						1.0	0.3

4.3 Photophysicochemical behaviour of MPc-particle conjugates

The photophysical studies were done for **7a**-ZnOMPs, **7a**-AgNPs, **7b**-AgNPs, and **8**-AgNPs.

4.3.1 Fluorescence spectra, lifetimes and quantum yields

The fluorescence emission, absorption and the excitation of complex **7a**-AgNPs and **7a**-ZnOMPs (as examples) are shown in **Figure 4.9**. For the conjugate of **7a** with ZnOMPs, the excitation band is slightly split, most likely due to lowering of symmetry on excitation (**Figure 4.9A**). The excitation spectrum of **7a**-AgNPs (**Figure 4.9B**) was red shifted and also split compared to the absorption spectrum. Red shifting of the excitation from the absorption spectrum indicate changes in the nuclear configuration upon excitation or differences in the packing of phthalocyanines on AgNPs [229,230]. Similar spectral changes were observed with **7b**-AgNPs and **8**-AgNPs. Due to the high degree of fluorescence quenching by the amino group combined with the heavy atom effect of silver nanoparticles, conjugate of **10** with AgNPs did not show any photophysical behaviour.

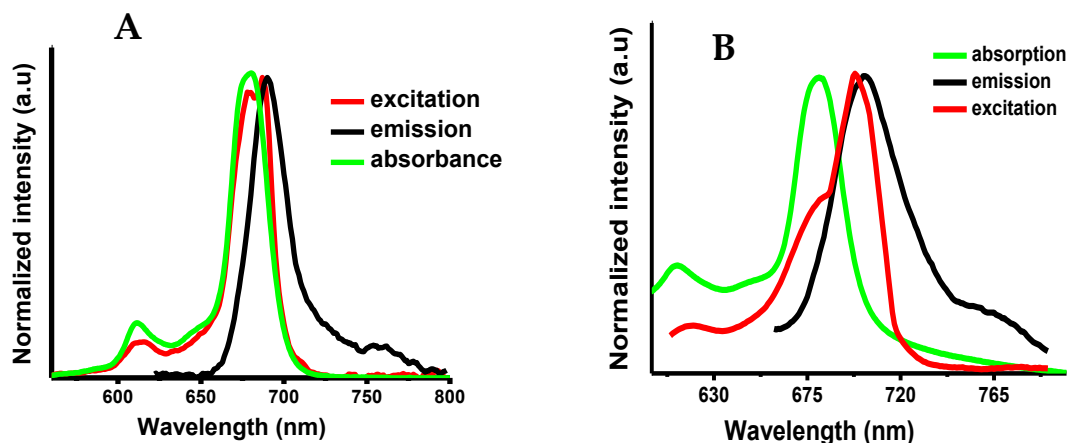


Figure 4.9: Absorption, excitation and emissions spectra of complex **7a**-ZnOMPs (A) and **7a**-AgNPs conjugate in DMSO (B).

The fluorescence quantum yields of the conjugates are shown in **Table 4.3**. The presence of AgNPs or ZnOMPs resulted in lower fluorescence quantum yields, **Table 4.3**. The low fluorescence quantum yields for the conjugates can be attributed to the heavy atom effect of the nanoparticles, which encourages the population of the triplet state and causes quenching of the fluorescence. The TCSPC method was used to determine fluorescence lifetimes of the conjugates. The lifetimes of the conjugates were obtained from the decay curves, **Figure 4.10**, which gave a bi-exponential fit, suggesting two different emitting species, while unconjugated phthalocyanines showed the presence of one fluorescent species.

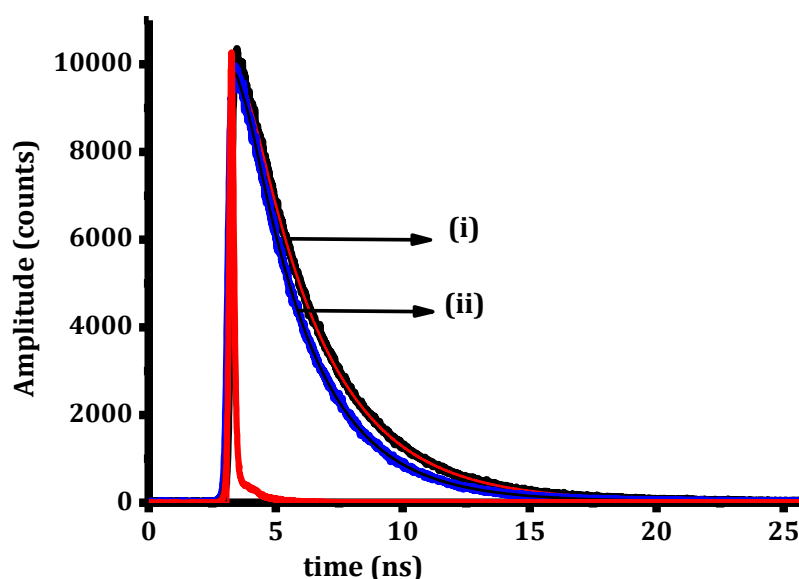


Figure 4.10: Time correlated single photon counting (TCSPC) fluorescence decay curves of **7b** (i), and **7b-AgNPs** (ii) in DMSO.

Time resolved emission spectra (TRES) was used to de-convolute the lifetimes of the conjugates and **Figure 4.11** shows TRES of **7b-AgNPs** (as an example). The two lifetimes corresponded to the emission that is due to unconjugated **7b** (709 nm, 2.83 ns), and the other unknown longer lifetime at 4.89 ns with emission at ~680 nm. We attribute the different lifetimes to the different arrangements of the Pc on the surface of silver nanoparticle. On conjugating nanoparticles to the phthalocyanine, the orientation or packing of the Pc onto the nanoparticles' surface may differ, resulting into multi-exponential decays [229,230]. Similar findings were observed with **7a-ZnOMPs** and **8-AgNPs**. However, with **7a-AgNPs**, although the TCSPC showed two lifetime, TRES only revealed single fluorescence lifetime that is similar to unconjugated **7a**.

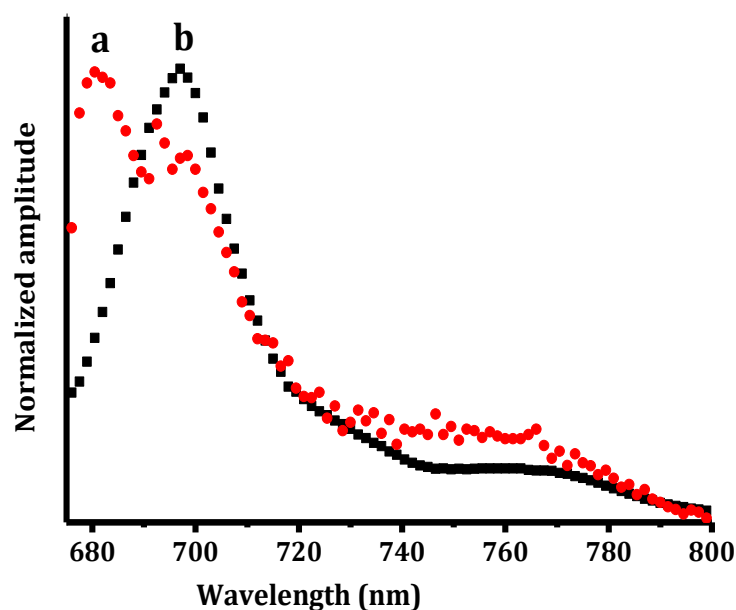


Figure 4.11: Time resolved emission spectra (TRES) of **7b**–AgNPs in DMSO. Emission at ~680 nm (**a**) is due to 4.89 ns and emission at 700 nm (**b**) is from 2.65 ns.

4.3.2 Triplet quantum yields and lifetimes

The triplet quantum yields and triplet lifetimes are listed in **Table 4.3**. Conjugating AgNPs to Pcs resulted in higher ϕ_T (compared to unconjugated Pc), this is attributed to the heavy atom effect (induced by the presence of nanoparticles) which encourages the ISC to the triplet state, as stated above. In general, larger ϕ_T are accompanied by shorter triplet lifetimes (τ_T) [231]. With the exception of **8**-AgNPs, in this work, larger ϕ_T were accompanied with longer lifetimes, **Table 4.3**. This could be due to the protection of the Pc by the AgNPs. The conjugate of **7a** with ZnOMPs resulted in lower triplet lifetimes; this is due to the fact that some of the excited electrons are injected into the valence band of ZnOMPs, therefore, reducing the number of photons populating the triplet state as shown in **Figure 1.2**

4.3.3 Singlet oxygen quantum yield studies

On conjugating complex **7a** to ZnOMPs, there was a decrease in the amount of singlet oxygen produced (from $\phi_{\Delta} = 0.61$ to 0.57). ZnOMPs have been reported to produce singlet oxygen. In this work, only the Q band of the Pc was excited, with the anticipation that some of the excited electrons will photosensitize ZnO, and therefore, result in an increased production of reactive oxygen species. We therefore deduce that the injected electron (**Figure 1.2**, step number **4**) did not have enough energy to excite the ground state molecular oxygen, however, other reactive oxygen species were produced as indicated by an increase in the photocatalytic behaviour in the presence of **7a**-ZnOMPs (which will be on page 147). Nonetheless, the presence of ZnOMPs slightly quenched the singlet oxygen. An increase in the ϕ_{Δ} values were observed upon conjugating silver nanoparticles to all Pc derivatives (**7a**, **7b**, **8** and **10**). These results are in agreement with the observed increased triplet state quantum yields upon conjugation (**Table 4.3**).

Table 4.3. Photophysicochemical parameters of complexes on conjugating to nanoparticles. Data was acquired in DMSO.

	ϕ_F	τ_F/ns^a	ϕ_T	$\tau_T (\mu\text{s})$	$\phi_\Delta (\text{a})$
7a	0.10	2.83 (1.00)	0.69	243	0.61 (0.25)
7a-AgNPs	0.06	2.89 (0.99)	0.71	271	0.69 (0.28)
		<0.01 (0.69)			
7a-ZnOMPs	0.10	2.82 (0.73)	0.66	233	0.57 (0.20)
		3.30 (0.27)			
7b	0.13	2.5 (1.00)	0.75	309	0.69 (0.26)
7b-AgNPs	0.04	2.65 (0.99)	0.85	359	0.80 (0.28)
		4.89 (0.061)			
8	0.21	3.48 (1.00)	0.59	412	0.48
8-AgNPs	0.15	3.55 (0.85)	0.60	399	0.50
		2.23 (0.15)			
10	0.015	2.6 (1.00)	0.50	99	0.31
10-AgNPs	-	-	-	-	0.36

(a) =values in brackets are singlet oxygen values in water for the immobilized Pc or Pc conjugates.

4.4 Photochemical behavior of supports: electrospun fibers and chitosan beads

The singlet oxygen of the functionalized fiber and beads was determined by the absolute method, with ADMA as the singlet oxygen quencher. The singlet oxygen quantum yields were determined in aqueous media, since the fiber or beads composites were going to be applied in water. **Figure 4.12** shows the degradation profile of ADMA using **7b** functionalized in polystyrene fiber and chitosan beads functionalized with **10**. As stated in the experimental section, singlet oxygen quantum yield values obtained are estimates when embedded into fibers or beads, they are however extremely useful for comparison purposes. The singlet oxygen quantum yields for **7a** and **7b** were determined to be 0.25 and 0.26, respectively, **Table 4.3**.

Singlet oxygen quantum yield values of 0.28, 0.20 and 0.28 were obtained for **7a**-AgNPs, **7a**-ZnOMPs and **7b**-AgNPs, respectively. As expected, the singlet oxygen quantum yields were higher upon conjugating silver nanoparticles as was observed in the studies done in organic solution, **Table 4.3**. Similar to results obtained in organic solution, the production of singlet oxygen by **7a**-ZnOMPs is lower compared to **7a** alone. The value for singlet oxygen of the functionalized beads was difficult to determine, as the UV-Vis absorption required for calculations (**Equation 1.5**) was impossible to obtain with microbeads. However, the decrease in the concentration of ADMA, 6.87×10^{-5} M at $t = 0$ to 5.91×10^{-5} M at $t = 50$ min, with an increase in

irradiation time, **Figure 4.12B**, is indicative that even though the Pc is confined in the beads, it can still produce singlet oxygen that is enough to degrade ADMA.

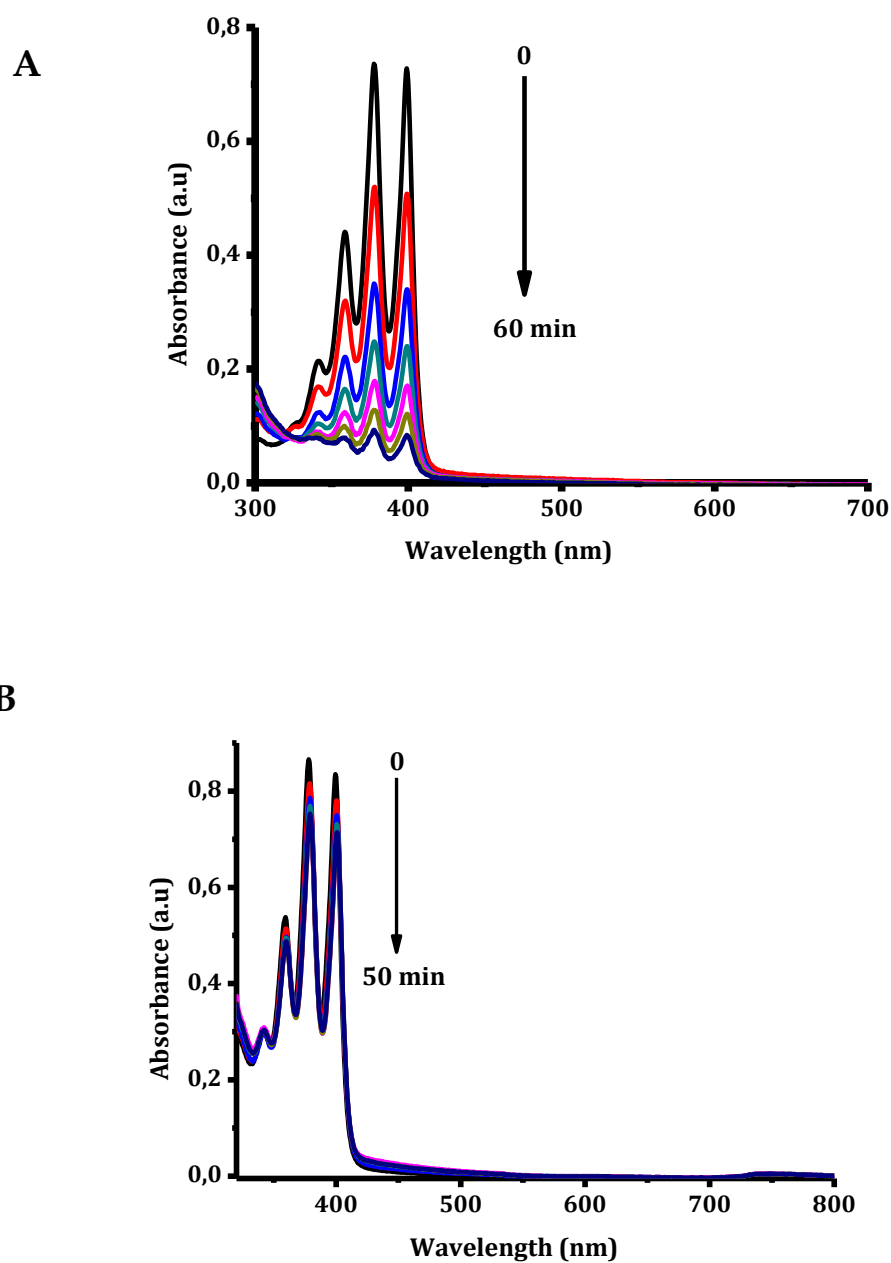


Figure 4.12: Degradation profile of ADMA ($\sim 3.33 \times 10^{-5}$ M) using **7b**-AgNPs/PS (A) and **10**-AgNPs/chitosan beads (B) in water (1 min interval).

CHAPTER 5

PHOTOTRANSFORMATION OF ORGANIC POLLUTANTS

The chapter discusses the photodegradation of organic pollutants using immobilized phthalocyanines and phthalocyanine-particle conjugates

5. Phototransformation of organic pollutants

Phthalocyanines can be employed as photocatalysts in solution or when dispersed in solid support systems [153]. In this work, the use of electrospun polymer fibers or chitosan microbeads functionalized with phthalocyanines and phthalocyanine-particle conjugates is explored for the degradation of environmental pollutants, Rhodamine 6G and methyl orange as model dyes. **Table 5.1** summarizes the photocatalysts used in the degradation of Rhodamine 6G or methyl orange.

Table 5.1: Summary of catalysts used in dye degradation

Abbreviation	Size of supports (μm)	Dye degraded
AgNPs/PS	3-8	methyl orange & Rh 6G
ZnOMPs/PS	3-6	Rh 6G
7a /PS	3-6	Rh 6G & methyl orange
7a -ZnOMPs/PS	3-6	Rh 6G
7a -AgNPs	3-8	methyl orange
7b /PS	3-6	methyl orange
7b -AgNPs/PS	3-8	methyl orange
10 / chitosan	452	Rh 6G
10 -AgNPs/ chitosan	452	Rh 6G

PS fiber = polystyrene fiber, Rh 6G = Rhodamine 6G

5.1. Photocatalytic degradation of Rhodamine 6G

5.1.1. UV-Vis spectral changes

The photocatalysts **7a**/PS, **7a**-AgNPs/PS, and **7a**-ZnOMPs/PS electrospun fibers were used in the phototransformation of Rhodamine 6G. Complex **10** and **10**-AgNPs were also functionalized onto chitosan beads and used as photocatalysts in the degradation of Rhodamine 6G. The effluent from the textile industry is discharged to the environment at different pHs. Thus, the efficiency of a photocatalyst needs to be examined under different pH conditions. In our study, two different pHs, acidic (pH 2) and basic (pH 9.2), were used to determine the photocatalytic activity of **7a**/PS, **7a**-

AgNPs/PS and **7a**-ZnOMPs/PS fibers. **Figure 5.1** shows the spectral changes of Rh 6G upon illumination in the presence of **7a**/PS (as an example), under acidic conditions. Similar spectral changes were observed with **7a**-ZnOMPs/PS or **7a**-AgNPs/PS, **10**-AgNPs/ chitosan or **10**/chitosan under basic (pH 9.2) and acidic (pH. As can be seen in **Figure 5.1**, the maximum absorption peak at 525 nm gradually decreased with time. The decrease in this peak was associated with a decrease in the fluorescence intensity as shown in **Figure 5.2**. There was no significant decrease of the Rh 6G peak on photolysis in the absence of the photocatalysts.

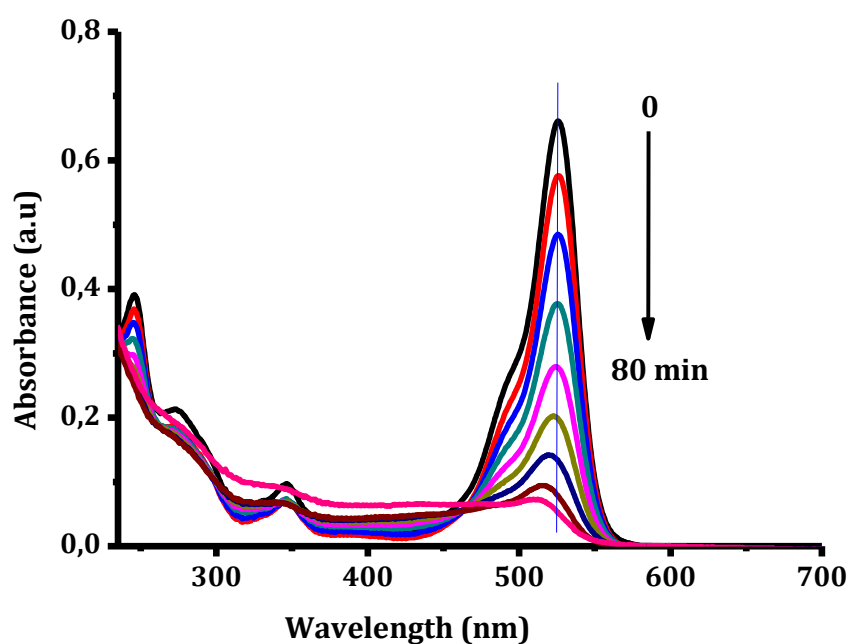


Figure 5.1: Spectral changes observed during the phototransformation of Rh 6G, with **7a**/PS as a catalyst under pH 2 conditions. Time interval = 10 min.

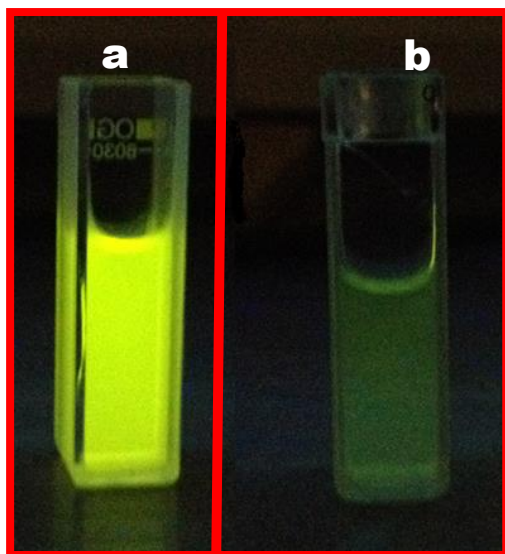
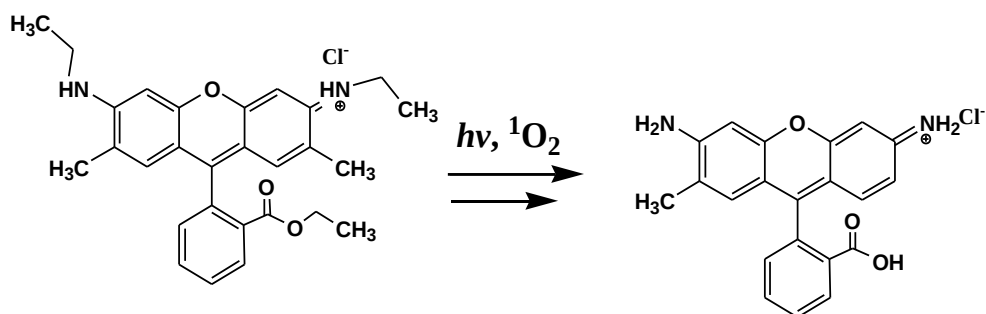


Figure 5.2: Decrease in Rhodamine 6G fluorescence with illumination in the presence of **7a** as a catalysts. **a)** Rhodamine 6G at $t=0$, and **b)** shows Rhodamine 6G after 60 min of irradiation.

There was also a noticeable blue shifting of the Rh 6G peak with time (**Figure 5.1**). According to Chen *et al.*, this blue shift in absorption spectra is related to the N-deethylation of Rh 6G [232].



Scheme 5.1: Deethylation of Rhodamine 6G by singlet oxygen produced upon excitation of the phthalocyanine.

Experiments where ZnOMPs/PS or AgNPs/PS were employed were also performed, however, there were negligible changes on the Rh 6G peak on excitation with visible

light since ZnOMPs absorbs strongly in the UV region. Irradiation in this study was done in the visible region only, so the photocatalytic behaviour is not expected for ZnOMPs. The role of AgNPs and ZnOMPs is to increase the production of reactive oxygen species (ROS), hence, increase the phototransformation rate of Rh 6G. Photolysis with electrospun polystyrene (without **7a**-ZnOMPs, **7a**-AgNPs or **7a** as photocatalysts) using visible light had no effect on Rh 6G transformation, the results indicate that the transformation of Rhodamine 6G is largely initiated by the reactive oxygen species produced upon exciting the phthalocyanine.

5.1.2. Reaction kinetics

5.1.2.1 Electrospun fibers

The plots of Rh 6G concentration *versus* time are shown in **Figure 5.3**. The kinetic data obtained from these plots is presented in **Table 5.2**. The linearity of the plots obtained, from the $\ln (C_0/C_t)$ *versus* time, indicate that the reactions followed the pseudo-first order kinetics. The rate (k_{obs}) of transformation of **7a**-AgNPs/PS was larger than that of **7a**/PS under both pH 2 and 9.2 conditions, when the concentration was 5.36 μM , **Table 5.2**. When the pH was 9.2, the presence of both particles (AgNPs or ZnOMPs) greatly enhanced the photocatalytic activity of **7a**-PS; this is attributed to the increased in the hydroxyl radicals at this pH [233]. In the presence of all photocatalysts, under acidic conditions, there was a reduction in the transformation rate of Rh 6G compared to pH 9.2 (**Table 5.2**). The major reduction in the reaction rates were observed with **7a**-

ZnOMPs/PS as a photocatalyst. The retarded efficiency at low pH is attributed to the photodissolution of ZnO under acidic conditions [234]. A half-life indicates the time it takes for half of Rhodamine 6G to be degraded, and the half-lives in the presence of **7a**, **7a**-ZnOMPs/PS, **7a**-AgNPs/PS were determined to be 52.9, 45.3 and 22.2 min, respectively, when the concentration of Rhodamine 6G was 5.13×10^{-6} M and the pH was 9.2. For all concentrations studied at this pH (pH 9.2), the half-lives were shorter in the presence of the conjugates and these were accompanied by faster reaction rates.

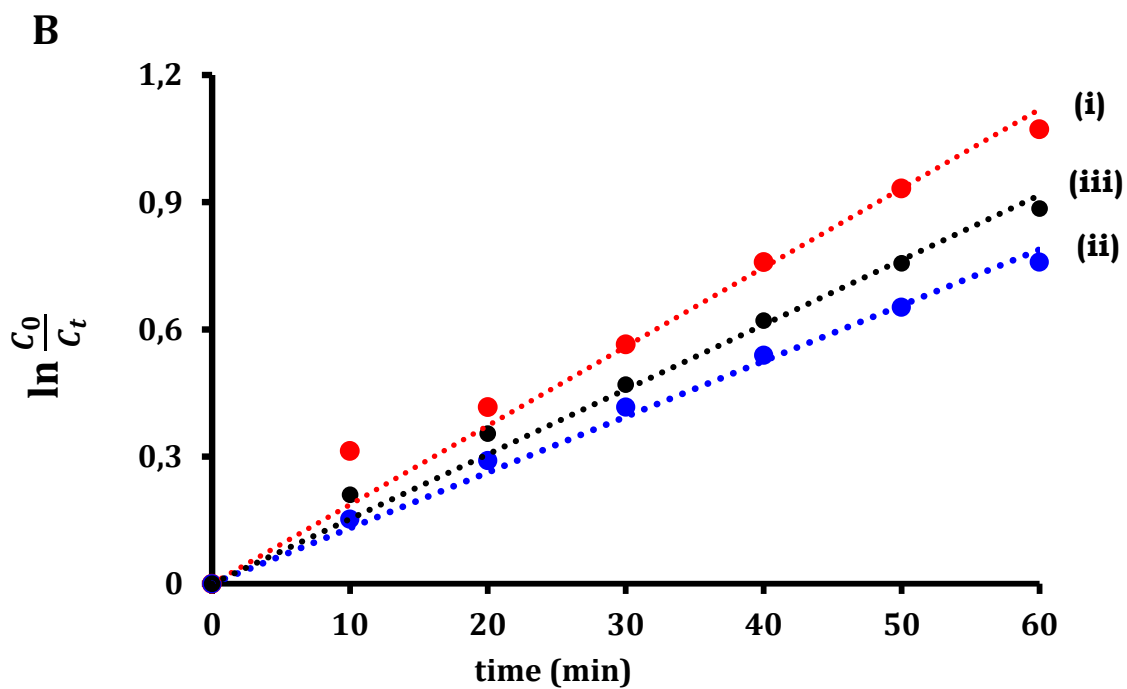
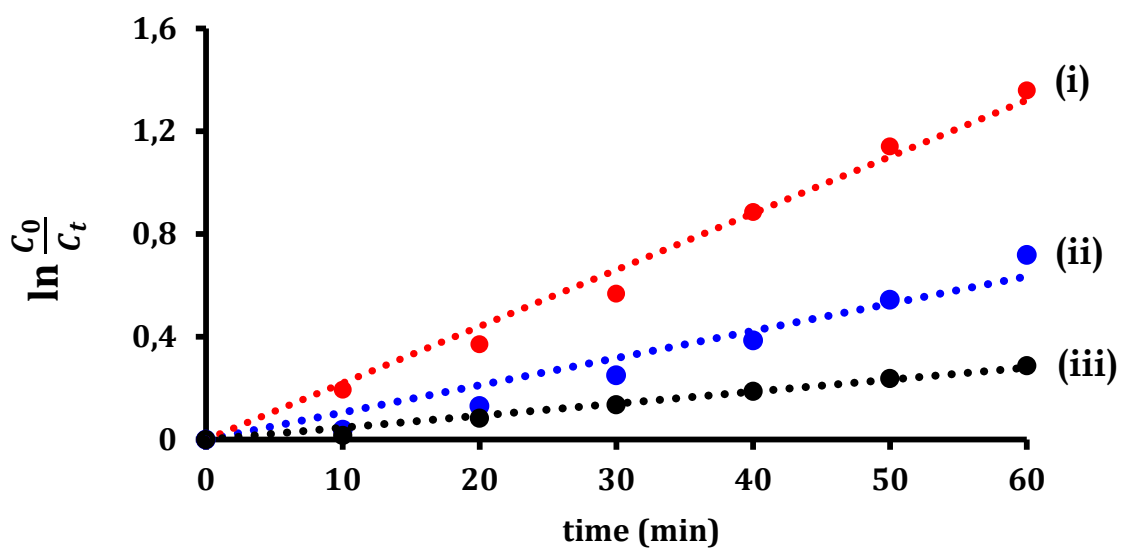


Figure 5.3: First order kinetics plots for the photodegradation of Rh 6G under pH 9.2 (A) and pH 2 conditions (B), with **7a**-AgNPs (i), **7a**-ZnOMPs (ii) and **7a** (iii) as photocatalysts.

Table 5.2: Kinetic data of transformation of Rh 6G using the unmodified and hybrid photocatalyst supported on polystyrene fibers. Excitation wavelength = 670 nm, Conc = 5.13 μ M.

	Φ_{Δ}	pH = 2			pH = 9.2		
		k_{obs} (min ⁻¹)	Initial-rate $\times 10^{-8}$ (mol L ⁻¹ min ⁻¹)	Half-life (min)	k_{obs} (min ⁻¹)	Initial-rate $\times 10^{-8}$ (mol L ⁻¹ min ⁻¹)	Half-life (min)
7a /PS	0.25	0.0105	5.71	66.0	0.0131	7.07	52.9
7a -ZnOMPs/PS	0.20	0.00467	2.51	148	0.0153	8.25	45.3
7a -AgNPs/PS	0.28	0.0189	10.18	36.6	0.03123	16.8	22.2

Studying the dependence of transformation efficiency on the initial dye concentration is of paramount importance from an applications point of view [235]. **Table 5.3** shows the effect of initial Rh 6G concentration on its photocatalytic transformation (pH = 9.2). In the presence of all prepared photocatalysts, the reaction rates (k_{obs}) decreased with an increase in the concentration of Rh 6G (**Table 5.3**). As the concentration of Rh 6G increases, the photons entering the solution are intercepted, therefore, reducing the phototransformation rates of Rh 6G at higher concentrations. The reverse was observed at lower concentration where there is an increase in the number of photons absorbed by the catalyst [236]. The same effect was observed by Wang *et al.* [236] during the photocatalytic decolorization of commercial dyes using zinc oxide powder as photocatalys. For all concentrations studied, an increase in the reaction rate, particularly in the presence of particles, was accompanied with shorter half lives.

Table 5.3: Kinetic data for PS fibers with various concentrations of Rhodamine 6G (pH=9.2).

Complex (a)	Concentration /μM	$k_{\text{obs}}/\times 10^{-2}$ min^{-1}	Initial rate/ $\times 10^{-8} \text{ M}^{-1}\text{min}^{-1}$	Half life /min
7a/Ps (0.25)	2.15	2.11	4.56	32.7
	3.44	1.68	5.78	41.3
	5.39	1.31	7.07	52.9
	8.70	0.934	8.13	74.2
	13.1	0.902	11.8	76.8
7a-AgNPs/Ps (0.28)	2.15	3.57	7.70	19.4
	3.44	3.55	12.2	19.5
	5.39	3.12	16.8	22.2
	8.70	2.58	22.4	26.9
	13.1	2.15	28.2	32.1
7a-ZnOMPs/Ps (0.20)	2.15	2.61	3.63	41.1
	3.44	2.34	5.08	46.9
	5.39	1.53	8.25	45.3
	8.70	1.15	9.99	60.3
	13.1	0.957	12.5	72.4

(a) represents the singlet oxygen quantum yields of the immobilized fiber in water.

The influence of initial dye concentration on the photocatalytic transformation in a heterogeneous system is described by the Langmuir–Hinshelwood kinetic model [237], Equation 5.1:

$$\frac{1}{r^0} = \frac{1}{kK_A} \frac{1}{C_0} + \frac{1}{k} \quad (5.1)$$

where k is the apparent reaction rate constant, K_A is the adsorption coefficient and C_0 corresponds to the initial concentration of Rhodamine 6G. Plots of the inverse of initial

reaction rate versus the reciprocal of the initial concentration of Rhodamine 6G were found to be linear with non-zero intercepts, **Figure 5.4**. The results presented in **Figure 5.4** give an indication that the Langmuir-Hinshelwood kinetic model is the appropriate model for describing the phototransformation of Rh 6G in the presence of the functionalized fibres. Therefore, it can be deduced that catalysis occurs at the surface of the supported photocatalyst. The adsorption coefficient (K_A) for all the functionalized fibres was determined from the slopes, **Figure 5.4**, and were found to be: 49.87, 24.15 and 31.76 mol⁻¹ L for **7a**/PS, **7a**-AgNPs/PS, and **7a**-ZnOMPS/PS, respectively, **Table 5.4**. A high adsorption coefficient indicates the tendency of the material to be adsorbed rather than remain in solution. The adsorption coefficient of **7a**-AgNPs/PS was lower compared to the other functionalised fibres, suggesting that desorption is much more favoured in this system. From the intercept in **Figure 5.4**, the apparent rate constants k were determined to be 1.29×10^{-7} , 6.45×10^{-7} and 2.02×10^{-7} mol L⁻¹min⁻¹ for **7a**/PS, **7a**-AgNPs/PS, and **7a**-ZnOMPS/PS, respectively, **Table 5.4**. The reaction rate was more enhanced by the presence of the AgNPs than ZnOMPs.

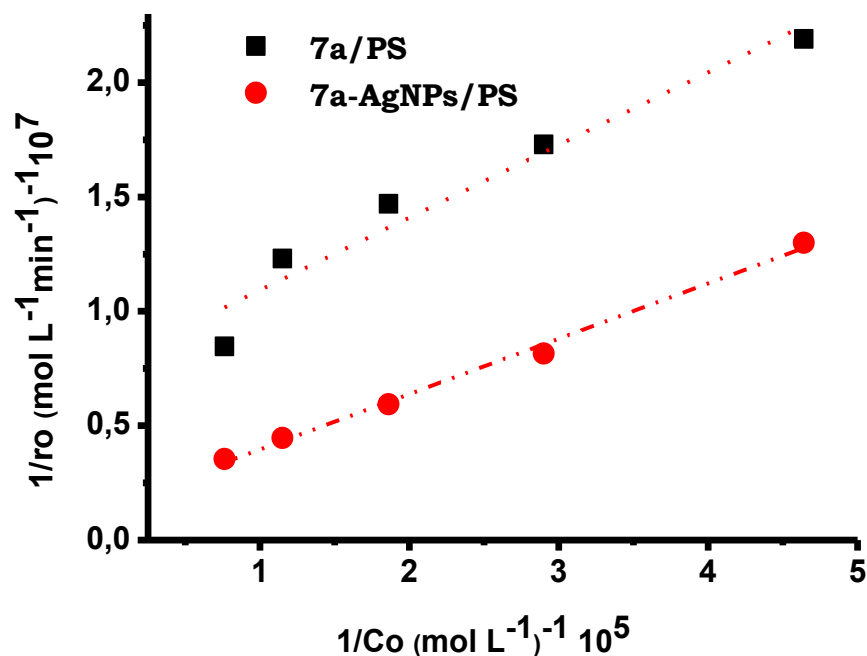


Figure 5.4: Plots of the reciprocal of the initial reaction rate versus the reciprocal of the initial concentration for transformation of Rhodamine 6G, pH 9.2.

Table 5.4: Langmuir-Hinshelwood parameters for the photocatalysis of Rh 6G with various catalysts.

Catalyst	$k \text{ (mol L}^{-1} \text{ min}^{-1}) \times 10^{-7}$	$K_A \text{ (mol L}^{-1})^{-1}$	ϕ_{Δ}^a
7a/PS	1.29	49.87	0.61
7a-AgNPs/PS	6.45	24.15	0.69
7a-ZnOMPs/PS	2.02	31.73	0.57
10/ chitosan	0.85	47.46	0.31
10-AgNPs/ chitosan	1.61	41.57	0.36

^a = singlet oxygen values of complexes in DMSO, and when not emdeded into supports.

5.1.2.2 Reaction kinetics of chitosan beads

Similarly to electrospun fibers, the linear plots of $\ln \left(\frac{C_0}{C_t} \right)$ *versus* time indicated that the degradation of Rhodamine 6G with **10** or **10**-AgNPs immobilized onto chitosan beads followed the pseudo-first order kinetics **Figure 5.5**, and the data obtained is shown in **Table 5.5**.

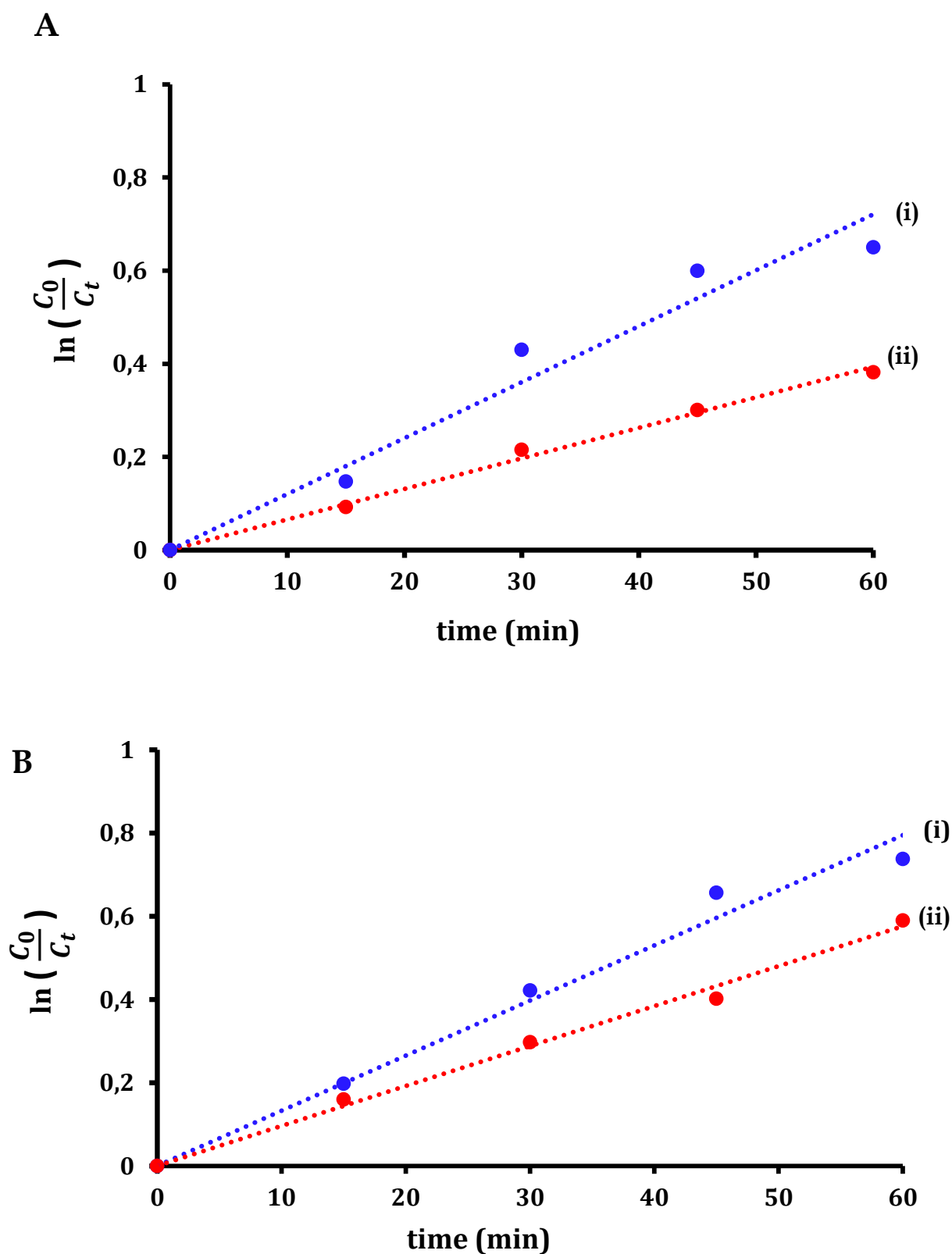


Figure 5.5: First order kinetics plots for the degradation of Rhodamine 6G at different concentrations with 10/chitosan (A) and 10-AgNPs/chitosan (B) beads. Initial concentration of Rhodamine 6G = 3.48×10^{-6} (i) 9.69×10^{-6} (ii).

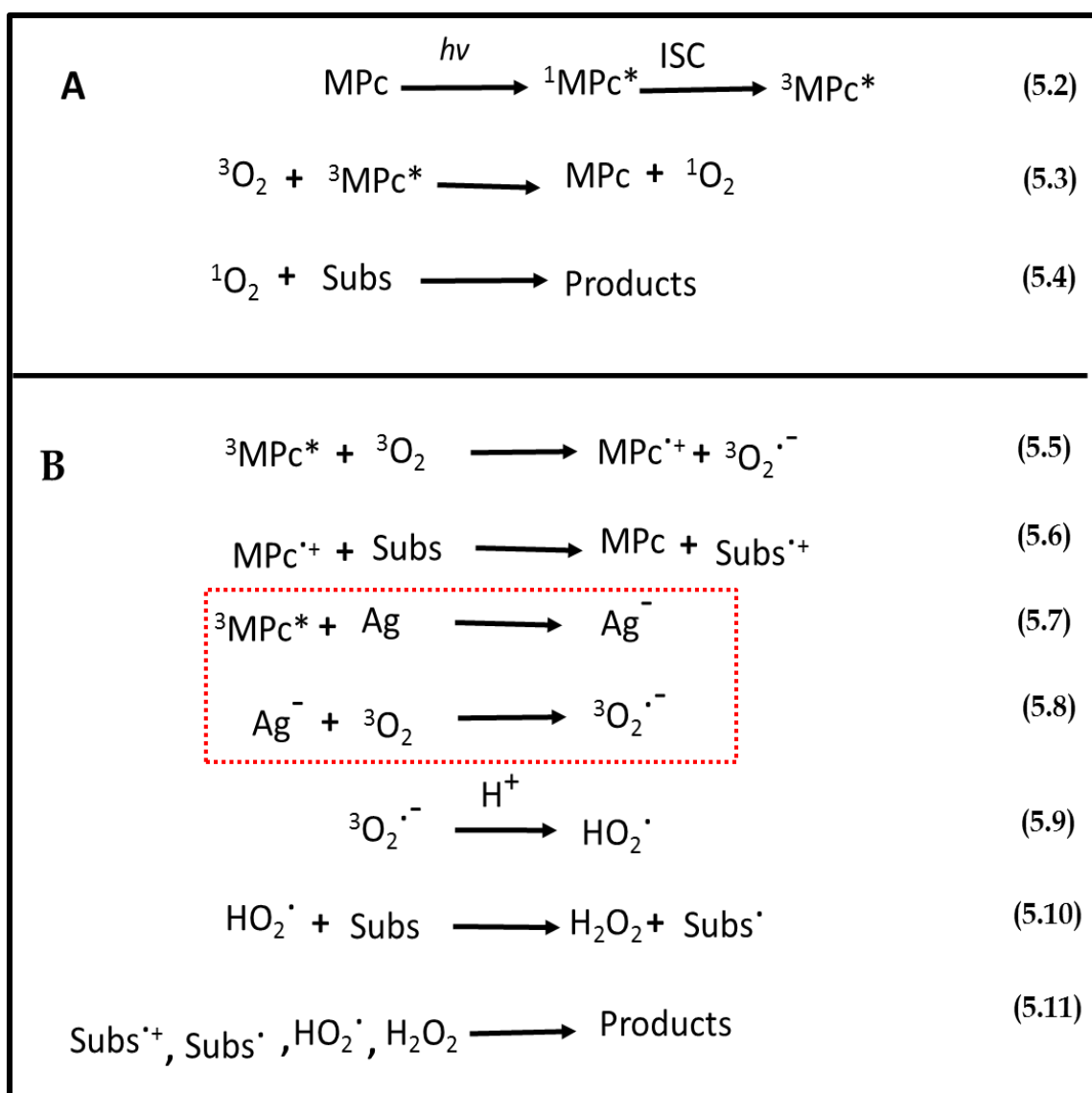
When the concentration of Rhodamine 6G was 5.13×10^{-6} M (as an example), the rate of degradation was found to be $k_{\text{obs}} = 9.61 \times 10^{-3}$ and $12.3 \times 10^{-3} \text{ min}^{-1}$ for **10**/chitosan and **10**-AgNPs/chitosan, respectively (Table 5.5). The half-lives for **10**/chitosan and **10**-AgNPs/chitosan were determined to be 72 and 56 min, respectively. For all concentrations studied, the half-lives were shorter for **10**-AgNPs/chitosan beads compared to the unconjugated **10**/chitosan, and the reaction rates (k_{obs}) were faster.

Table 5.5: Kinetic data for PS fibers with various concentrations of Rhodamine 6G (pH=9.2).

Compound	Φ_{Δ}^a	Conc ($\times 10^{-6}$ M)	k_{obs} ($\times 10^{-3}$ min^{-1})	Initial-rate ($\times 10^{-8}$ $\text{mol L}^{-1} \text{ min}^{-1}$)	Half-life (min)
10/ chitosan	0.31	2.18	15.2	3.31	45.66
		3.48	11.8	4.09	58.94
		5.13	9.61	4.93	72.13
		9.69	6.92	6.09	110.2
		20.1	3.85	7.74	179.9
10-AgNPS/ chitosan	0.36	2.18	17.1	3.73	40.53
		3.48	13.3	4.61	52.31
		5.13	12.3	6.33	56.20
		9.69	9.32	9.03	74.34
		20.1	9.56	13.2	105.6

*a= singlet oxygen values for **10** or **10**-AgNPs in DMSO, and when not supported on chitosan beads.*

Reactive oxygen species are formed *via* two processes, type I and type II, **Scheme 5.2**. While both mechanism are possible, research has shown that in the presence of phthalocyanines, type II (**Scheme 5.2**, Eqs. 5.3 - 5.4) is the dominant process. Type I (**Scheme 5.2**, Eqs. 5.5- 5.11) is the reactive oxygen species formation, which reacts with Rhodamine 6G, therefore, causing the degradation of the dye.



Scheme 5.2: Type II (A) and type I (B) photosensitization of Rhodamine 6G with immobilized **10** and **10**-AgNPs. Sub represents substrate which is Rhodamine 6G.

The production of singlet oxygen by the beads was confirmed with ADMA as a singlet oxygen quencher as described in **Section 4.4**. Irrespective of the marginal increase in the singlet oxygen quantum yield of **10** after conjugating AgNPs, an increase in the reaction rate was observed in the presence of silver nanoparticles, **Table 5.5**. This indicates that, other than type II, type I mechanism was also involved in the degradation of the dye. When **10** is used as a photocatalyst, with the exception of **Eqs. 5.7** and **5.8** (**Scheme 5.2**), both type I and type II mechanisms are plausible, and can result into the degradation of Rhodamine 6G. However, in the presence of Ag, in addition to the heavy atom effect, Ag can also produce more superoxide that reacts with the dye (**Scheme 5.2**, **Eqs. 5.7** and **5.8**), which ultimately results into the observed increase in the photocatalytic reaction in the presence of AgNPs.

Langmuir–Hinshelwood (L-H), **Equation 5.1**, was also used to assess the extent of adsorption of Rhodamine 6G onto the beads (**Figure 5.6**). Results obtained from Langmuir–Hinshelwood plots are listed in **Table 5.4**. The apparent rates (k) were determined from the intercepts, and were found to be 8.51×10^{-8} , and 16.10×10^{-8} mol L⁻¹min⁻¹ for **10**/chitosan or **10**-AgNPs/chitosan, respectively. The reaction rate was highly enhanced by the presence of nanoparticles as was observed with **7a**. Adsorption coefficients (K_A) for both functionalized beads were determined from the slopes, and were found to be 41.57 and 47.46 mol⁻¹L for **10**/chitosan and **10**-AgNPs/chitosan, respectively, **Table 5.4**. A higher adsorption coefficient was observed for **10**/chitosan showing that adsorption is more favoured in the absence of AgNPs, the same was observed for **7a**. The apparent rates are higher for **7a**, and **7a**

conjugates compared to **10** and **10** conjugates, **Table 4.3**; this could to be related to the high singlet oxygen quantum yields produced by **7a**, **Table 5.4**. This then demonstrates the importance of singlet oxygen in photocatalysis.

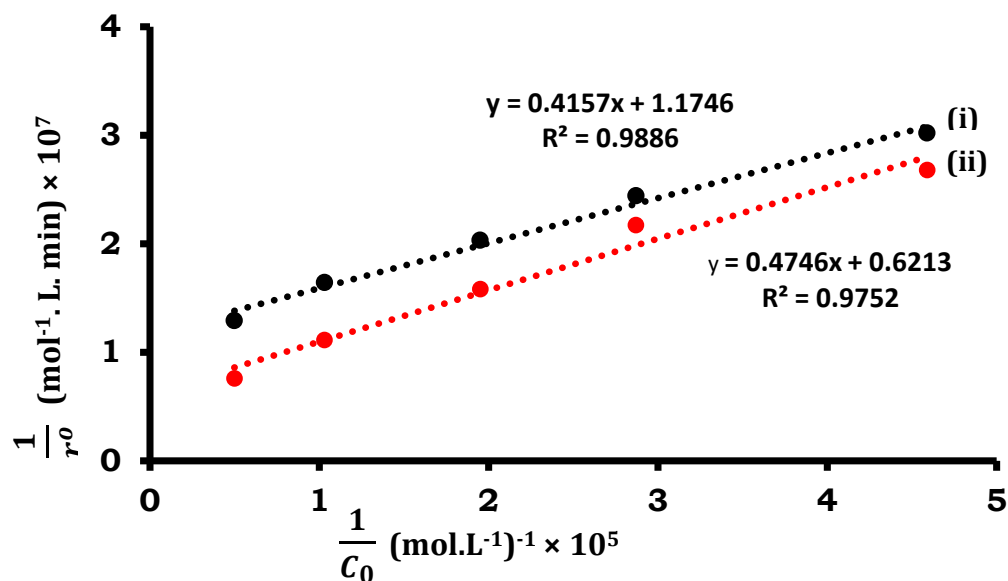


Figure 5.6: Plots of the reciprocal of the initial reaction rate versus the reciprocal of the initial concentration for the degradation of Rhodamine 6G with **10**-AgNPs/chitosan (i) and **10**/chitosan (ii) beads as photocatalysts.

5.1.3 Chromatographic analysis

The products of phototransformation were characterized using HPLC, and the chromatograms are shown in **Figure 5.7(A)**. The chromatogram in **Figure 5.7(A)** shows the appearance of new peaks at 8 and 11 min, where the appearance of these new peaks is accompanied by a decrease in the main Rh 6G peak at 12 min. UV-Vis spectra of the phototransformation products at $t = 11$ or 8 min (**Figure 5.7B**) are similar to that of Rh 6G ($t=12$ min). However, there exists clear hypsochromic shifts of the

transformations products from the parent Rh 6G peak. This blue shifting has been associated with the N-deethylation of Rhodamine [232], **Scheme 5.1**. Rhodamine derivatives are reported to undergo deethylation prior to complete mineralization of the dye [232].

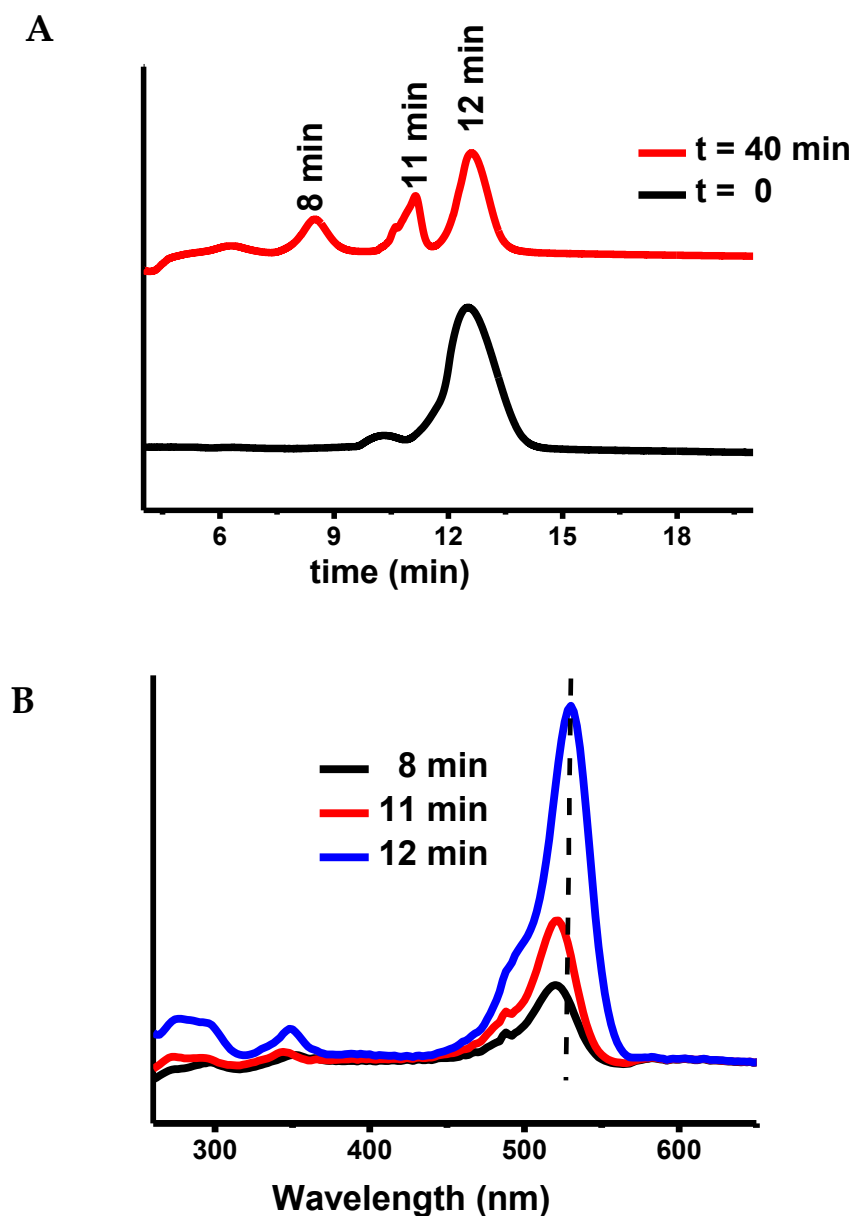


Figure 5.7: HPLC chromatograms (A) of phototransformation products of Rh 6G obtained at $t = 0$ and after 40 min irradiation time, and the UV-Vis spectra (B) of the peaks corresponding to 8, 11 and 12 min in the HPLC spectrum obtained in $t = 40$ min. The photocatalysts employed was **7a**/PS at pH 9.2.

5.1.4 Catalyst stability and recyclability

The stability of the functionalized catalyst (using **7a**/PS) was examined for the phototransformation of Rh 6G during a five cycle experiment, **Figure 5.8**. Prior to each cycle, the catalyst was washed several times with water and dried at room temperature. **Figure 5.8** shows the percentage phototransformation of Rh 6G using **7a**/PS as an example. The % dye phototransformation with time was calculated using **Equation 5.12**:

$$\% \text{ Photodegradation} = \frac{C_0 - C_t}{C_0} \times 100 \quad (5.12)$$

where C_0 and C_t are Rh 6G concentration before and at time t after photolysis. **Figure 5.8** shows that photocatalytic activity remained relatively unchanged after each cycle, there were no significant changes on the % dye transformation. This confirms the stability and efficiency of **7a**/PS for the transformation of Rh 6G under visible light irradiation, and that the catalyst could be easily separated for re-use. Similar behavior was observed for all the other photocatalysts used in the degradation of Rhodamine 6G.

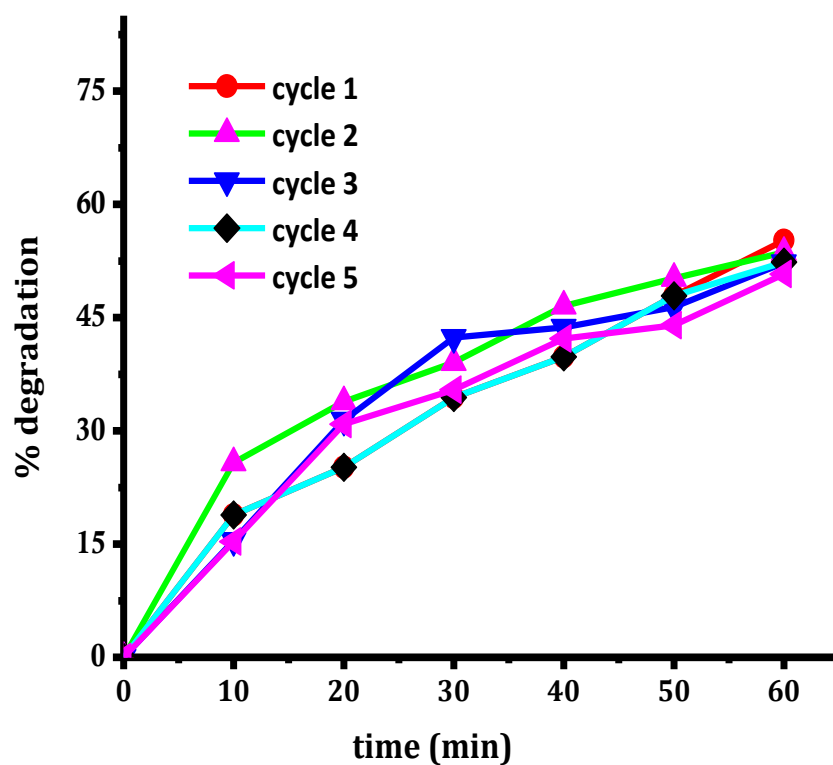


Figure 5.8: Percentage Rh 6G removal, under pH 2 conditions, in the presence of the unmodified photocatalysts (7a/PS).

5.2 Photocatalytic degradation of methyl orange

5.2.1 UV-Vis spectral changes

Methyl orange was also used as a model dye, photocatalysts **7a**/PS, **7a**-AgNPs/PS, **7b**/PS, **7b**-AgNPs/PS were used in the photodegradation of this dye. Different concentrations of methyl orange were irradiated for 30 min time intervals, and the spectral changes were monitored at 470 nm. The strong absorption band at 470 nm is attributed to the absorption of the $n-\pi^*$ transition related to the azo bond [238], **Figure 5.9** (for **7b**-AgNPs/PS as an example). There was a significant decrease of this peak with an increase in the irradiation time, indicating the degradation of methyl orange and the breaking of the azo bond. Similar spectral changes were observed when **7a**/PS, **7a**-AgNPs/PS and **7b**/PS were used as catalysts. There were no changes in the absorption band of methyl orange in the absence of the phthalocyanine photocatalysts, implying that the degradation of methyl orange is initiated by its reaction with the reactive oxygen species generated by the Pc.

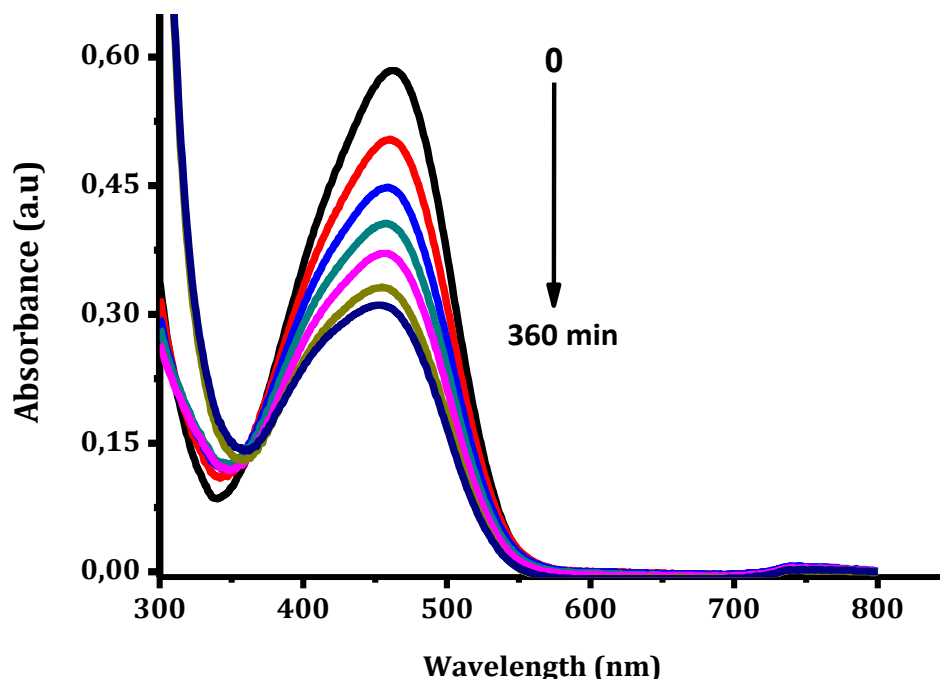


*Figure 5.9: UV-Vis spectral changes of an aqueous solution of methyl orange (pH=9.2 , Initial conc. of methyl orange = 2.31×10^{-5} M) with **7b**-AgNPs/PS.*

An enhancement of photocatalysis in the presence of AgNPs is well reported in literature, particularly when photocatalysts such as TiO_2 or Fe_3O_4 are employed [239,240]. Research has also shown that there is enhancement of the photocatalytic activity of silver halide microspheres in the presence of AgNPs; this increase in the degradation rate is referred to as plasmonic photocatalysis [241-243]. In the current work, silver nanoparticles (without Pc) embedded in electrospun fibers were also used as photocatalysts in the degradation of methyl orange, and there was an insignificant decrease on the methyl orange peak upon illumination with visible light.

5.2.2 Reaction kinetics

The photocatalytic activity of the immobilized photocatalysts was evaluated by comparing their apparent kinetics behaviour. Similar to Rhodamine 6G, the linear plots of $\ln \left(\frac{C_0}{C_t} \right)$ vs time (with different concentrations) were obtained, indicating pseudo-first order kinetics for the immobilized catalysts, **Figure. 5.10**. These findings correlates with those reported in literature [26,244,245]. The reaction rate, initial rate, and half-lives were determined from these plots, and the results are tabulated in **Table 5.6**. There was a decrease in the rate of the photodegradation (k_{obs}) of methyl orange with an increase in concentration as observed for Rh 6G.

Non-peripherally substituted phthalocyanine (**7b**/PS) showed higher reaction rates than peripherally substituted counterpart (**7a**/PS). The reaction rates, when the concentration of methyl orange was 1.00×10^{-5} M, were found to be $k_{obs} = 0.00256$ and 0.00497 for **7a**/PS and **7a**-AgNPs/PS, respectively. The time it takes for half ($t_{1/2}$) of methyl orange (concentration= 1×10^{-5} M) to be consumed was determined to be 270 and 139 min for **7a**/PS and **7a**-AgNPs/PS, respectively. The half-lives obtained are comparable to literature values reported for the photodegradation of methyl orange [245].

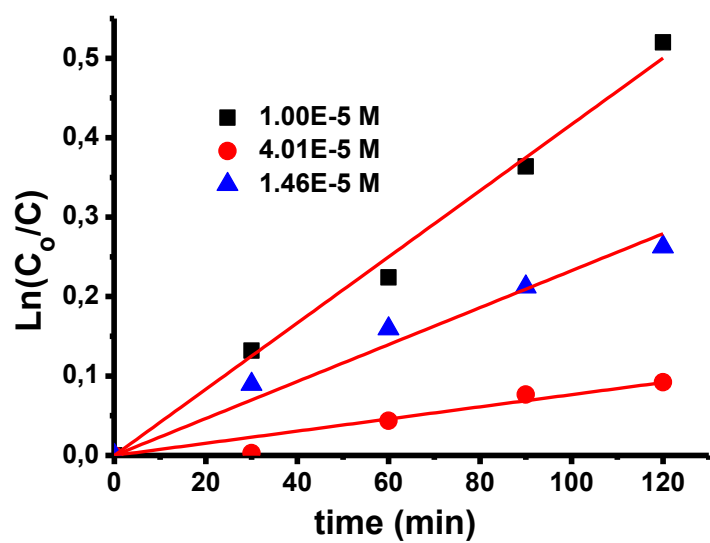


Figure 5.10: First order kinetics plots for the degradation of methyl orange at different concentrations with 7b/PS. Studies were done in water at pH 9.2.

Table 5.6: Kinetic data for PS fibers with various concentrations of methyl orange (pH=9.2).

Compound	Φ_{Δ}	Conc ($\times 10^{-5}$ M)	k_{obs} ($\times 10^{-3}$ min)	Initial-rate $\times 10^{-8}$ (mol L $^{-1}$ min $^{-1}$)	Half-life(min)
7a/PS	0.25	1.00	2.56	2.56	270
		1.46	1.85	2.70	374
		2.31	1.30	3.00	533
		4.01	0.795	3.19	871
		5.26	0.616	3.24	1125
7a-AgNPs/PS	0.28	1.00	4.97	2.01	139
		1.46	4.36	1.57	158
		2.31	3.36	1.29	206
		4.01	2.85	0.875	243
		5.26	2.06	0.923	336
7b/PS	0.26	1.00	3.74	3.74	185
		1.46	3.13	4.57	221
		2.31	2.53	5.84	273
		4.01	2.22	8.90	312
		5.26	2.06	10.8	336
7b- AgNPs/PS	0.28	1.00	3.91	3.91	177
		1.46	2.85	4.16	243
		2.31	1.96	4.53	353
		4.01	1.29	5.17	537
		5.26	1.00	5.26	693

The results obtained from the linear plots of the LH expression (**Figure 5.11**, **7a** and **7a-AgNPs** as examples) were used to determine the apparent rate (k) as well as adsorption coefficients of the photocatalysts. The apparent rates were determined to be 1.84×10^{-8} , 6.58×10^{-8} , 11.1×10^{-8} and 16.8×10^{-8} mol L $^{-1}$ min $^{-1}$ for **7a/PS**, **7a-**

AgNPs/PS, **7b**/PS and **7b**-AgNPs/PS, respectively (Table 5.7). Adsorption coefficients (K_A) for all functionalised fibers were found to be 314.9, 294.6, 526.6 and 413.2 mol⁻¹ for **7a**/PS, **7a**-AgNPs/PS, **7b**/PS and **7b**-AgNPs/PS, respectively. The adsorption coefficients of **7a** or **7b** were higher compared to their conjugates. However, for all photocatalyst, the adsorption coefficients were greater than one suggesting that desorption is much more favoured in this systems. When comparing the degradation of Rhodamine 6G to that of methyl orange, with **7a** as a photocatalyst, the rates of degradation are much faster with Rh 6G. This is attributed to the greater stability of the azo dye compared to the xanthene dyes [191-193].

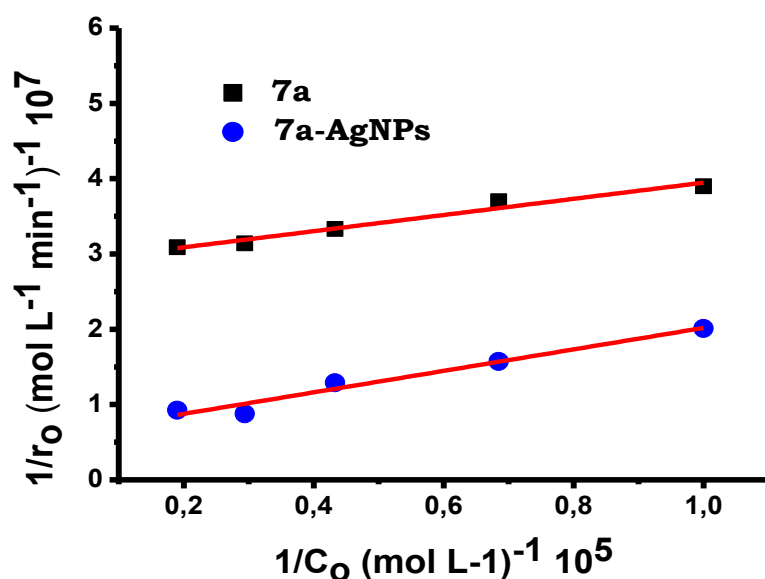


Figure 5.11: Plots of the reciprocal on initial reaction rate versus the reciprocal of the initial concentration for degradation of methyl orange. Studies were done in water at pH 9.2.

Table 5.7: Langmuir-Hinshelwood parameters for the photocatalysis of methyl orange
Studies were done in water at pH 9.2.

Catalyst	$k \text{ (mol L}^{-1} \text{ min}^{-1}) \times 10^{-8}$	$K_A \text{ (mol L}^{-1})^{-1}$
7a/PS	1.84	314.9
7a-AgNPs/PS	6.58	294.6
7b/PS	11.1	526.6
7b-AgNPs/PS	16.8	413.2

CHAPTER 6

PHOTODYNAMIC THERAPY OF CANCER

This chapter discusses the photodynamic activity of phthalocyanines when conjugated to nanoparticles (Ag) or targeting molecules (folic acid and polylysine).

6.1. Photodynamic therapy of phthalocyanines: *In vitro* cell studies

Some phthalocyanines, e.g. Pc-4, are already in clinical trials for treatment of different malignancies. Pc-4 is a complex formulation that is insoluble in water. Thus, the search for phthalocyanines that are water soluble, have defined composition and show improved PDT activity, is continuing. Hence, our research focuses on Pc photosensitizers soluble in aqueous media with improved PDT selectivity. We have demonstrated that conjugating a Pc to folic acid or polylysine improve the solubility of phthalocyanines in aqueous media (**10**-FA, **11**-polylysine). The other strategy employed (in this work) for inducing water solubility of the Pc is the quaternization of the nitrogen, the water soluble Pc is then conjugated to silver nanoparticles (**8**-AgNPs) to improve toxicity. In this chapter, *in vitro* photodynamic activity of the prepared conjugates (**8**-AgNPs, **10**-FA and **11**-polylysine) is discussed.

6.1.1 PDT of **8** in the presence of AgNPs on melanoma cancer cell line.

Silver nanoparticles were added to complex **8** to enhance the selectivity of complex **8** toward cancerous cells. Complex **8** has two types of donor atoms located on the ring substituent: nitrogen and oxygen. However, the lone pair of the nitrogen atom is expected to be engaged as a result of quaternization, and those of the ring are sterically hindered by the benzene rings of the isoindole, this leaves the lone pairs of oxygen free for any interaction with AgNPs.

Therefore, the interaction between the nanoparticles and the Pc is proposed to be via the lone pairs of oxygen. It is possible, depending on the wavelength employed, for silver nanoparticles to provide plasmonic enhancement of PDT. The cell studies in this work were performed by irradiating complex **8** alone or in the presence of AgNPs at a wavelength of 676 nm, where only the former absorbs. The SPR band of AgNPs employed in this work is too high in energy to be involved, **Figure 3.15**. The cytotoxicity of a photosensitizer is an essential aspect in PDT as it gives important information about the efficiency of a photosensitizer. A good photosensitizer should kill cells in the presence of light only. The neutral red uptake assay provides a quantitative estimation of viable cells, the method has been described as a sensitive assay to evaluate viable cells in a monolayer culture [246], and was used in this study to determine % viability.

The dark toxicity of **8** in the presence or absence of nanoparticles was determined, **Figure 6.1**. Initially, 2 mL (20 %) and 1 mL (10 %) of colloidal silver nanoparticles were mixed with complex **8**. However, the mixture was highly cytotoxic without any irradiation at this concentration of silver, while 5 % AgNPs showed the least cytotoxicity as shown in **Figure 6.1**. Cell death at 80 μ M of complex **8** + AgNPs (5%) was found to be 23 %, while complex **8** alone killed 16 % of the cells (**Figure 6.1**). Concentrations lower than 80 μ M resulted in a decrease in cell death as expected; thus, the results obtained indicate that the photosensitizer concentrations used have little cytotoxicity in melanoma cancer cell in the absence of light. The cytotoxicity of

complex 8 was enhanced by the presence of AgNPs, this is attributed to the well-known toxicity of AgNPs [15,16].

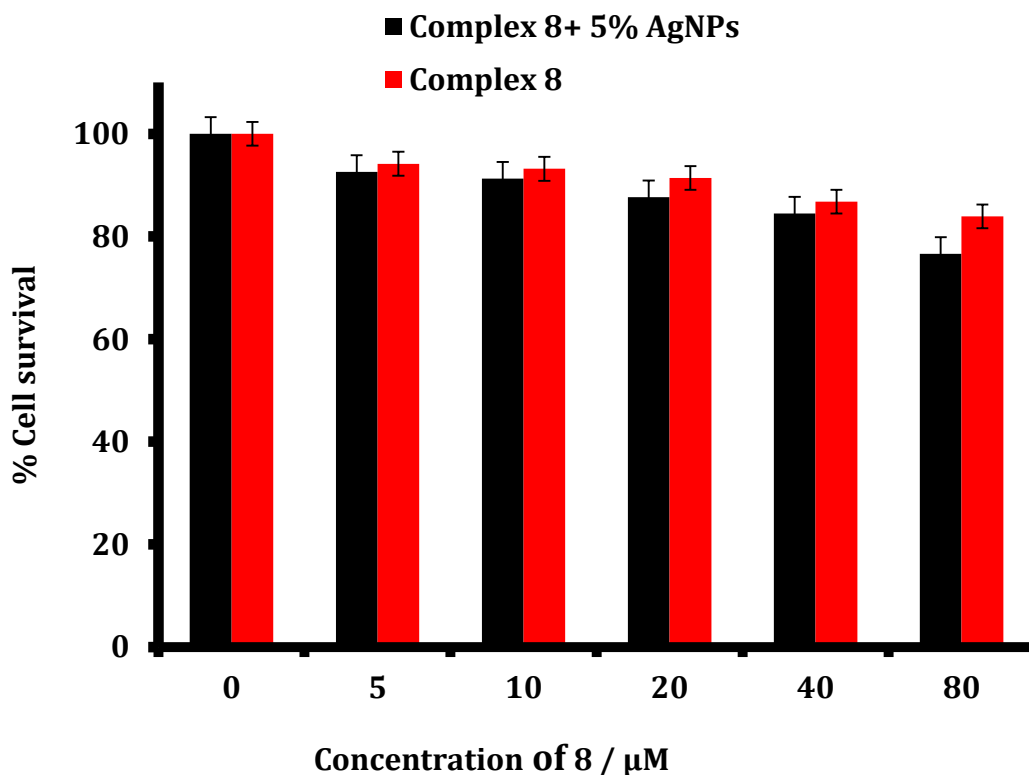


Figure 6.1: Dark cytotoxicity of complex 8 and complex 8 + 5% AgNPs. Data represents the mean values from three independent experiments. Error bars are standard deviations.

Low-level laser therapy (LLLT) is reported to aggravate skin cancer, while laser therapy is well known in the treatment of cancer [247,248]. The appropriate laser dose is required to ensure that the laser is not responsible for the cell death nor does it promote cell growth. This will in turn ensure that normal cells are not affected during photodynamic therapy. The laser doses investigated showed little effect on the cells, with 5 J/cm² killing only 11 % of the cells, **Figure 6.2**. Therefore, 2.5 J/cm² was used

for the activation of different concentrations of complex **8** with or without nanoparticles.

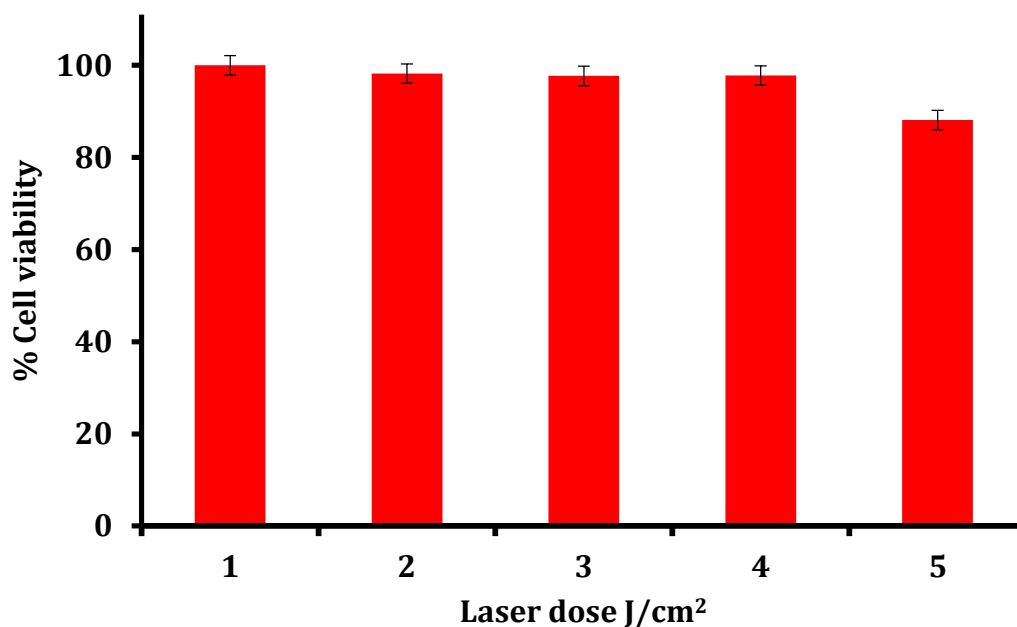


Figure 6.2: Laser dose toxicity on melanoma cancer cell lines. The data represent the mean values from three independent experiments. Error bars are standard deviations.

An inverted microscopy was used to determine morphological changes of the cells after PDT. The images in **Figure 6.3** show cells treated with 80 μ M of complex **8** or with 80 μ M of **8** + 5% AgNPs before (**A** and **B**) after PDT (**C** and **D**). Viable or living cells attach to the surface of a dish. Upon treating cells with 80 μ M of complex **8**, with or without nanoparticles, there was no significant change in the cellular morphology in the dark. After PDT, the cells acquired a round shape, losing their normal shape and they were also found to be detached for both unconjugated complex **8** and

complex **8** with AgNPs. These observations are typical morphological changes associated with apoptosis [249].

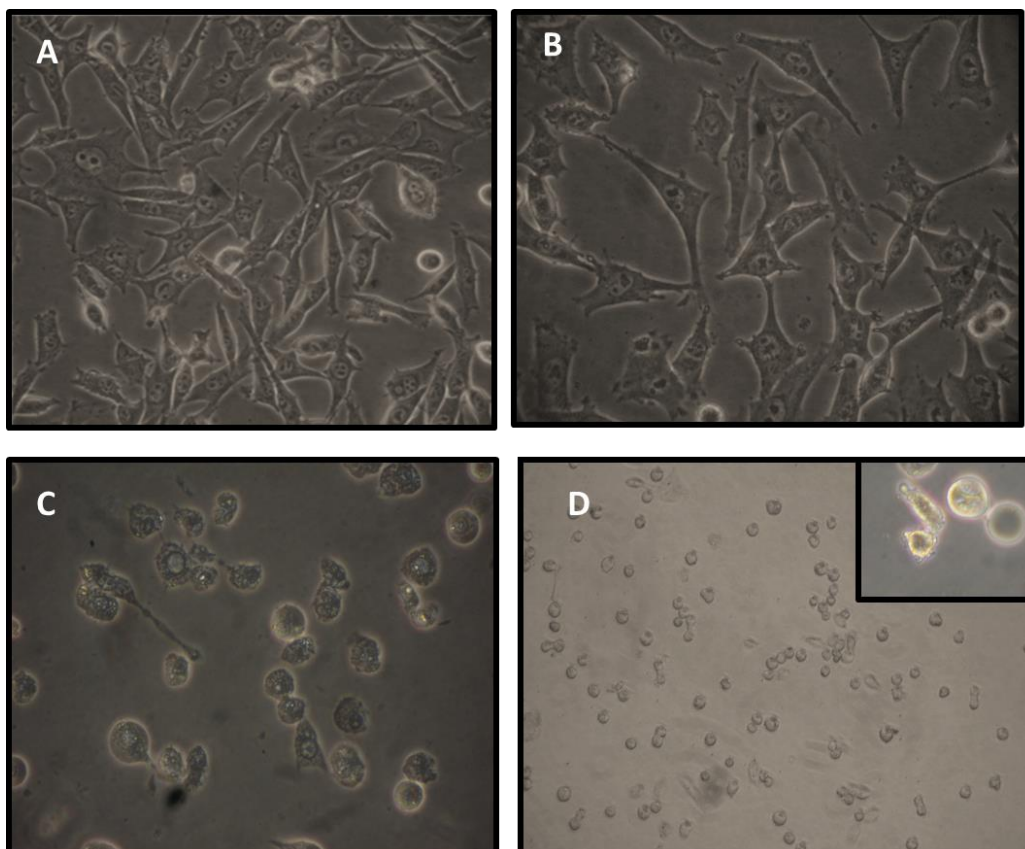


Figure 6.3: Images of melanoma cancer cells after incubating for 24 hr with **8** (A) and **8**+ 5 %AgNPs (B) in the dark. C and D show cells incubated for 24 hr with **8** and **8** + 5% AgNPs, respectively, and irradiated with 2.5 J/cm².

Changes in morphology cannot be used to evaluate the potency of the treatment. The neutral red viability assay was used to quantify the fraction of viable cells. On irradiating melanoma cells with laser light in the presence of at 80 μ M of complex **8**, 45% of the cells survived while 24 % of the cells survived in the presence of 5%

nanoparticles (i.e. complex **8** with AgNPs), **Figure 6.4**. In the presence of silver nanoparticles (for all concentrations tested), the cell death increased when compared to cells treated with complex **8** alone. Complex **8** was aggregated in water, and the aggregates were highly photoinactive. Therefore, the singlet oxygen quantum yield of **8** was only determined in organic media (**Table 4.3**). The presence of silver nanoparticles marginally increased the production of singlet oxygen, this combined with the toxicity of Ag increased the cellular death in the presence of silver nanoparticles. Similar findings have been reported when Zhou *et al.* chelated Au and Pt to hypocrellin, where an improvement in *in vitro* PDT was observed in the presence of these heavy metals [250]. Lower cell survival rates (~10%) have been reported for melanoma cells in literature using hydrophobic ClAlPc [251], but using much larger concentrations (order of 10^{-4} M) of the photosensitizer compared to order of 10^{-5} M employed in this study.

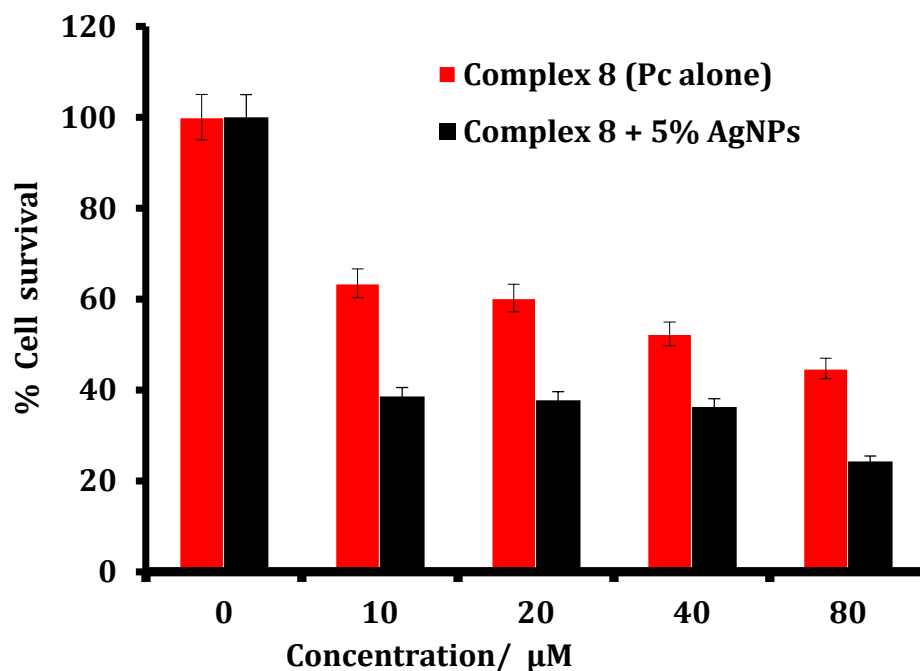


Figure 6.4: Photodynamic cytotoxicity of complex 8 and complex 8 + 5% AgNPs. Irradiation dose = 2.5 J/cm^2 . Data represent the mean values from three independent experiments. Error bars are standard deviations

6.1.2 PDT of 10 and 10-folic acid conjugate on MCF-7 breast cancer cell line

In this study, the efficacy of a photosensitizer, zinc tetra-amino phthalocyanine, when conjugated to folic acid is reported, it is anticipated that folic acid will improve the selectivity of **10**. As mentioned previously, photosensitizers are designed to be non-toxic to cells in the absence of light, but exhibit toxicity when illuminated with the appropriate wavelength of light [252,253]. The cytotoxicity or dark toxicity of **10** and **10-FA** was examined and **Figure 6.5** shows the % cell survival after incubating MCF-7 cells with different concentrations of the **10** and when conjugated to folic acid. As the graph indicates, without light, **10** or **10-FA** showed no dark toxicity to cells, except

for 360 μM which showed cellular death ($\sim 20\%$) in the presence of both tested compounds. It is also worth noting that cellular survival in the presence of **10**-FA is slightly higher than that of the **10** alone, we attributed this to the presence of folic acid which is required for cellular growth and replication.

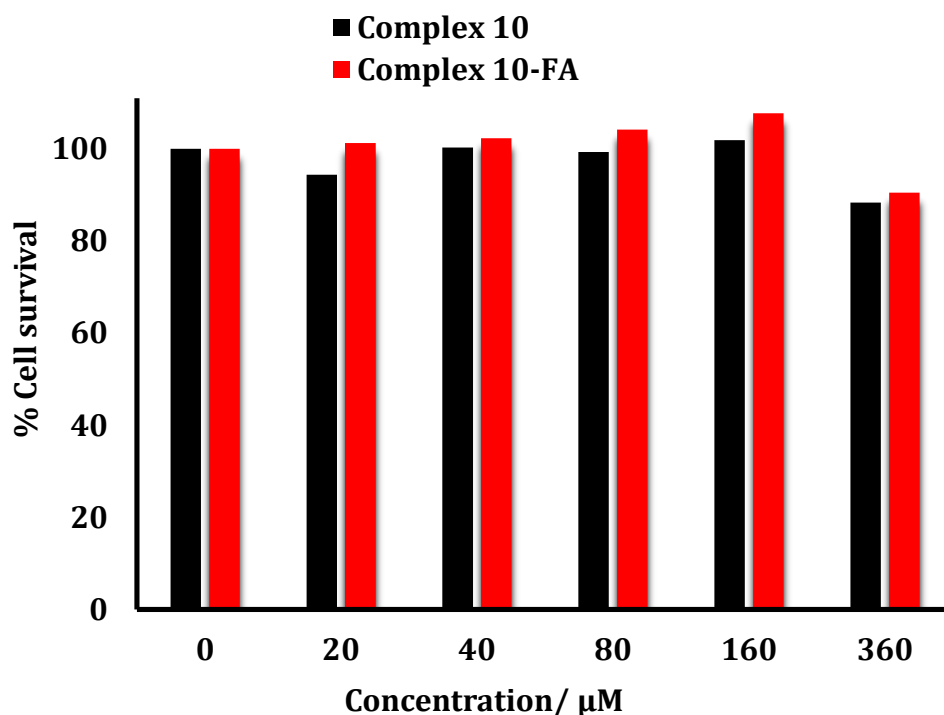


Figure 6.5: Percentage survival of MCF-7 cells when incubated with different concentrations of **10** and **10**-FA for 24 hours under conditions detailed in the experimental section.

A combination of light and **10** or **10**-FA resulted in decreased cellular viability when compared to the control with no photosensitizer. **Figure 6.6A** and **B** show light and concentration dependent decrease in the cellular viability, in the presence of **10** or **10**-FA. In the presence of **10**-FA (160 μM), cellular viability of 48, 34 and 32 % was obtained when the light density of 22.9, 145.8 and 91.6 J/cm^2 was used, respectively. Whereas with **10** (160 μM), cellular viability of 54, 52 and 41 % was attained when the

light dose of 22.9, 145.8 and 91.6 J/cm² was used, respectively. The efficacy of the conjugate was higher than that of **10** alone. We have shown the reduction in singlet oxygen production upon conjugating to folic acid, although the conjugate has a lower singlet oxygen quantum yield ($\phi_{\Delta} = 0.21$, **Table 4.2**) compared to **10** ($\phi_{\Delta} = 0.31$, **Table 4.2**), the conjugate exhibited greater photodynamic activity when compared to the Pc alone. When the concentration was 160 μ M and the light dose of 91.6 J/cm² was used, 59 % and 68 % cell death was observed when **10**-FA and **10** were employed, respectively. It has been reported that the photophysical properties of a photosensitizer does not necessarily always provide an accurate indication of the phototoxicity of the PS [254,255]. The photo-toxicity of the photosensitizer is defined by a number of factors including cell type, cellular uptake and localization [256]. Nonetheless, **10**-FA possess greater photodynamic activity than **10**. This is presumable due to the increase in cellular uptake of **10**-FA, which is largely facilitated by the presence of folic acid.

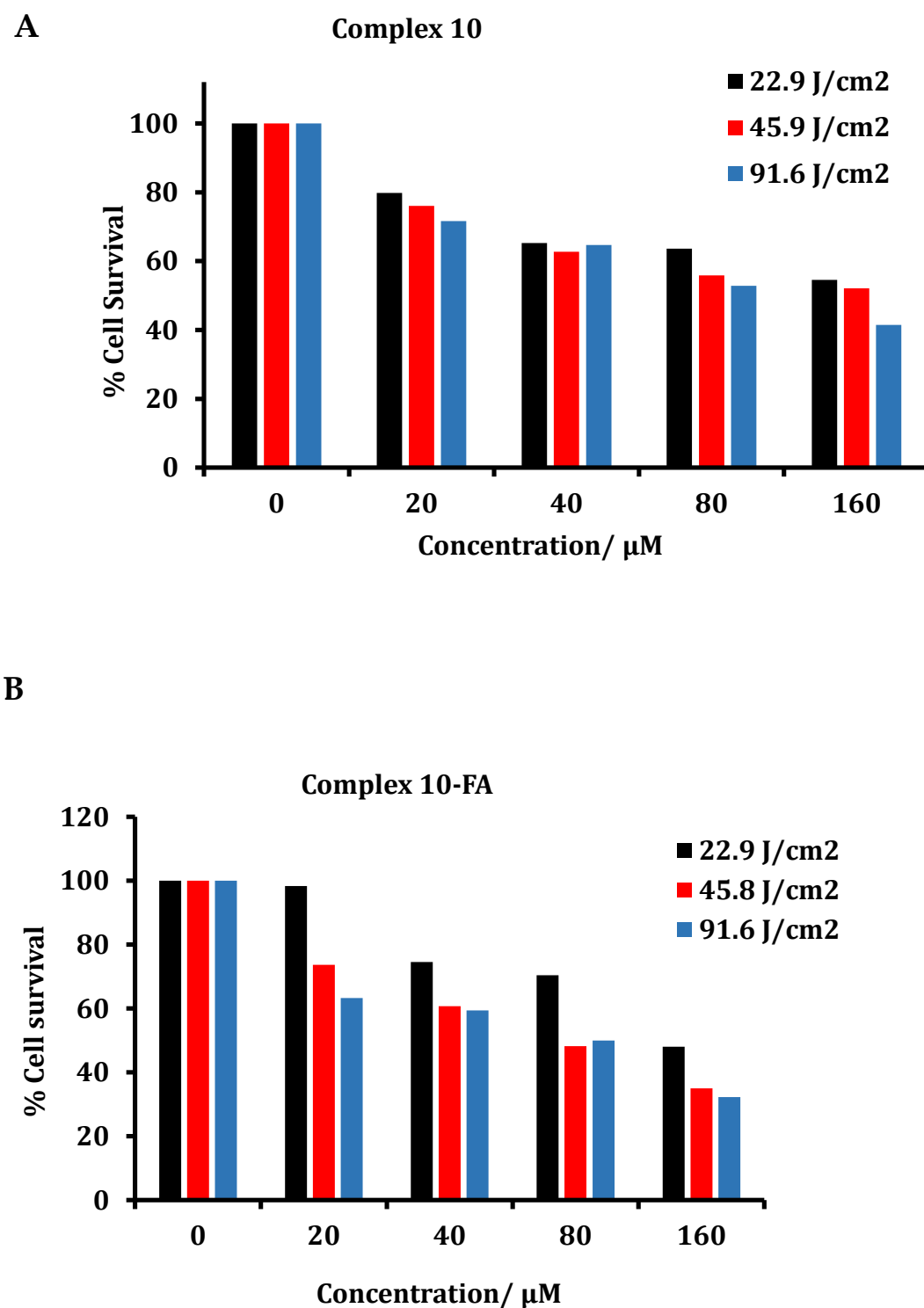


*Figure 6.6: Photodynamic inactivation of MCF-7 cells with **10** (A) and **10-FA** (B) illuminated with power densities of 22.9, 45.8 and 91.6 J/cm²*

Morphological changes of cells associated with photodynamic therapy were investigated using an inverted microscopy. The images on **Figure 6.7** show cells treated with 160 μM of **10**-FA before (**A**) and after irradiation (**B**) with 91.6 J/cm^2 . On treating cells with 160 μM , there was no significant change on the cells morphology (**Figure 6.7A**). After PDT cells became round losing their normal shape (**Figure 6.7B**), and they were also de-attached from the bottom of the plate; showing typical apoptotic morphological changes [249]. Similar morphology changes were obtained after PDT in the presence of **10**.

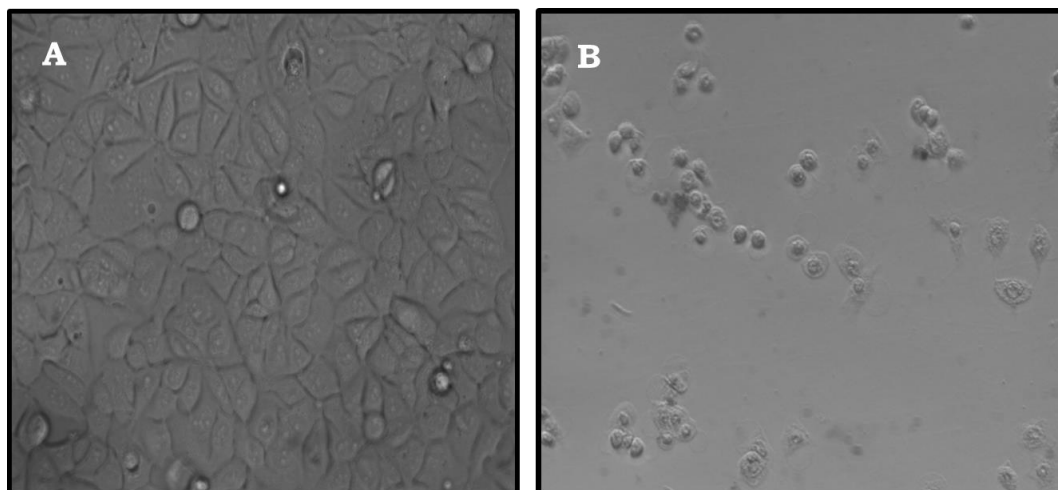


Figure 6.7: Micrographs of MCF-7 cells in the presence of 160 μM of **10**-FA before (A) and after irradiation (B) with a power density of 91.6 J/cm^2 .

6.1.3 PDT of **11** and **11** polylysine conjugate on prostate cancer cell line

Cell studies reported in this section were undertaken in Geneva, Switzerland. Due to time constraints, the study was not repeated and other studies such as dark toxicity were omitted. However, the results obtained after PDT give substantial findings that can be used as a basis for future studies when polylysine is conjugated to phthalocyanines. Prostate cancer cells lines were subjected to light in the presence of different concentrations (1, 2, 4 and 8 μM) of **11**- PLL conjugate and unconjugated **11**. **Figure 6.8** depicts the results obtained after irradiating cells containing the conjugate and **11**. As the concentration of **11** is increased, there is no significant effect of the drug on the cells, the percentage cell survival is almost the same in all concentrations except for 1 μM of the Pc where the survival is lower compared to other concentrations. This might be from an experimental error. However, with the conjugates, as the concentration of the conjugate is increased, there is a consistent decrease in the cell survival.

The solubility between **11** and **11**-PLL was different; unconjugated **11** was soluble in DMSO and other organic solvents, and the conjugate (**11**-PLL) was water soluble, but insoluble in organic solvents. The difference in cellular uptake can be attributed to the varying degree of solubility of **11** and the conjugate. The cells absorbed more of the water soluble conjugate and little amount of **11**, this accounts for the high percentage survival of the cells in the presence of **11**. These are preliminary findings, reproducibility of the data will still need to be determined.

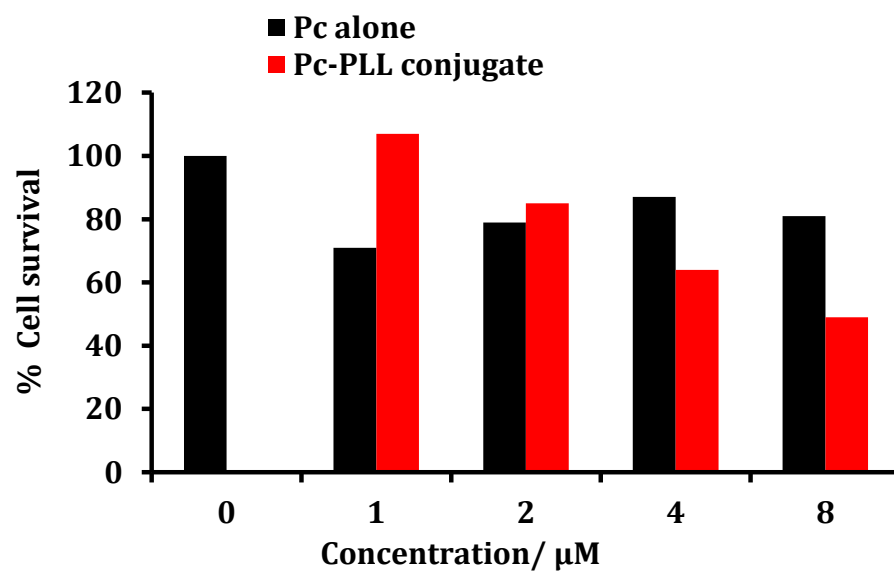


Figure 6.8: Percentage cell survival of irradiated PC-3 cells with 11 and 11-polylysine conjugates.

CHAPTER 7

CONCLUSIONS AND FUTURE PROSPECTS

7.1 Conclusions

This work presents the successful synthesis and characterization of new phthalocyanines functionalized at the non-peripheral and peripheral positions. The prepared phthalocyanines showed improved solubility in common organic solvents, and were found to exhibit monomeric behaviour in solution. We also described the effect of silver nanoparticles and ZnOMPs on the photochemical and photophysical behaviour of tetra substituted phthalocyanines. With the exception of ZnOMPs, the presence of nanoparticles (AgNPs) enhanced the singlet oxygen and triplet quantum yields of the phthalocyanines; this was attributed to the heavy atom effect of the nanoparticles.

The conjugates were then immobilized onto electrospun fibers and chitosan microbeads for use in heterogeneous photocatalysis. The interaction between particles, phthalocyanines and supports does not restrict the formation of the excited triplet state since relatively high singlet oxygen quantum yields were obtained in aqueous media. Photocatalysis of the immobilized conjugates was successfully evaluated with methyl orange and Rhodamine 6G as model dyes. The presence of both ZnOMPs and AgNPs (under basic pH) showed improved photocatalytic behaviour compared to individual photocatalysts. Therefore, in this study, we have demonstrated that water insoluble phthalocyanines can act as active photocatalysts for the oxidation of organic pollutants, with enhanced catalytic rate in the presence of

nanoparticles. Furthermore, we have successfully conjugated phthalocyanines to folic acid and polylysine with the aim of using these conjugates in the eradication of cancer. The high degree of solubility of these conjugates in aqueous media makes them good photosensitizers for PDT. The potency of the conjugates towards different cancer cells was demonstrated. The conjugates were found to exhibit negligible dark toxicity, with increased cellular growth observed when cells were incubated with the conjugate of folic acid. Although these new photosensitizers were highly aggregated in water, they still showed reasonable potency towards cancer cells.

7.2 Future Prospects

Polylysine conjugate to phthalocyanine should be explored further. It can make an interesting study particularly when conjugated to phthalocyanines with the aim of forming a pro-drug, where the photosensitizing abilities of the conjugate is “switched on” once inside the cells. The other aspect that needs further exploration is the preparation of chitosan spheres immobilized phthalocyanines, although we have demonstrated that immobilizing phthalocyanines onto chitosan does not compromise the photocatalytic behaviour of the phthalocyanine, it will be interesting to study the effect of phthalocyanine loading on the beads. Since chitosan is also used in removing metals, perhaps a dual catalyst can be prepared where metals are introduced along with organic dyes. The cells studies of MPc-ZnO conjugate were not conducted in this study, in future, it will be interesting to conduct *in vitro* cell studies of the conjugate just

to confirm that the observations made during photocatalysis are indeed maintained even in biological applications.

REFERENCES

1. C. Havilla, E. Ciliberto, Inorganic nanoparticles: Synthesis, Applications and perspective, CRC Press, 2010.
2. M.A Malik, P. ÓBrien, N. Revaprasadu, Phosphorus Sulfur 180 (2005) 689.
3. G. Schmid, Nanoparticles from theory to application, Wiley-VCH Verlag, 2nd Ed., Weinheim, 2010.
4. A. Henglein, M. Giersig, J Phys Chem B 103 (1999) 9533.
5. T. Maneerung, S. Tokura, R. Rujiravanit, Carbohydr Polym 72 (2008) 43.
6. K. Madhumathi, P.T. Sudheesh Kumar, S. Abhilash, V. Sreeja, H. Tamura, K. Manzoor, S. V. Nair, R. Jayakumar, J Mater Sci: Mater Med 21 (2010) 807.
7. T.A. Dankovich, D.G. Gray, Environ Sci Technol 45 (2011) 1992.
8. W.-L. Chou, D.-G. Yu, M.-C. Yang, Polym Adv Technol 16 (2005) 600.
9. H. Karadeniz, A. Erdem, A. Caliskan, C.M. Pereira, E.M. Pereira, J.A. Ribeiro, Electrochem Commun 9 (2007) 2167.
10. J.M. Köhler, L. Abahmane, J. Wagner, J. Albert, G. Mayer, Chem Eng Sci 63 (2008) 5048.
11. A. Avalos, A.I. Haza, D. Mateo, P. Morales, J Appl Toxicol 34 (2014) 413.
12. C. Carlson, S.M. Hussain, A.M. Schrand, L.K. Braydich-Stolle, K.L. Hess, R.L. Jones, J.J. Schlager, J Phys Chem B 112 (2008) 13608.

BIBLIOGRAPHY

13. O. Choi, Z. Hu, *Environ Sci Technol* 42 (2008) 4583.
14. K.M.M. Abou El-Nour, A. Eftaiha, A. Al-Warthan. R.A.A. Ammar, *Arab J Chem* 3 (2010) 135.
15. R. Foldbjerg, P. Olesen, M. Hougaard, D.A. Dang, H.J. Hoffmann, H. Autrup, *Toxicol Lett* 190 (2009) 156.
16. I. Sur, D. Cam, M. Kahoursaman, A. Baysal, M. Culha, *Nanotechnology* 21 (2010) 175104
17. T. Stuchinskaya, M. Moreno, M.J. Cook, D.R. Edwards, D.A. Russell, *Photochem Photobiol Sci* 10 (2011) 822.
18. D. Bechet, P. Couleaud, C. Frochot, M.-L. Viriot, F. Guillemin, M. Barberi-Heyob, *Trends Biotechnol* 26 (2008) 612.
19. D.K. Chatterjee, L.S. Fong, Y. Zhang, *Adv Drug Delivery Rev* 60 (2008) 1627.
20. Q.H. Tran, V.Q. Nguyen, A.-T. Le, *Adv Nat Sci: Nanosci Nanotechnol* 4 (2013) 1.
21. C.N.R. Rao, P.J. Thomas, G.U. Kulharni, *Nanocrystals: Synthesis, Properties and Applications*, Springer, Germany, 2007, 10.
22. A. Kołodziejczak-Radzimska, T. Jesionowski, *Materials* 7 (2014) 2833.
23. I. Gonzalez-Valls, M. Lira-Cantu, *Energy Environ Sci* 2 (2009) 19.

BIBLIOGRAPHY

24. J. Liqiang, S. Xiaojun, S. Jing, C. Weimin, X. Zili, D. Yaoguo, F. Honggang, *Sol Energy Mater Sol C* 79 (2003) 133.
25. A. Mills, S. Le Hunte, *J Photochem Photobiol A: Chem* 108 (1997) 1.
26. M.A. Chamjangali, S. Boroumand, *J Braz Chem Soc* 24 (2013) 1329.
27. Z.L. Wang, *J Phys Condens Matter* 16 (2004) R829.
28. Ü. Ozgur, D. Hofstetter, H. Morkoc, *Pr Inst Electr Elect* 98 (2010) 1255.
29. L. Wang, Y. Kang, X. Liu, S. Zhang, W. Huang, S. Wan, *Sensor Actuator B* 162 (2012) 237.
30. H. Zhang, B. Chen, H. Jiang, C. Wang, H. Wang, X. Wang, *Biomaterials* 32 (2011) 1906.
31. N. Padmawathy, R. Vijayaraghavan, *Sci Technol Adv Mater* 9 (2008) 035004.
32. S. Gosh, V.S. Goudar, K.G. Padmalekha, S.V. Bhat, S.S. Indi, H.N. Vasan, *RSC Adv* 2 (2012) 930.
33. H. Yin, P.S. Casey, M.J. McCall, M. Fenech, *Langmuir* 19 (2010) 15399.
34. L. Spanhel, M. A. Anderson, *J American Chem Soc* 113 (1991) 2826.
35. Q. Zhong, E. Matijević, *J Mater Chem* 6 (1996) 443.

BIBLIOGRAPHY

36. K. Sue, M. Suzuki, K. Arai, T. Ohashi, H. Ura, K. Matsui, Y. Hakuta, H. Hayashi, M. Watanabe, T. Hiaki *Green Chem* 8 (2006) 634–638.
37. A. K. Gangul, S. Vaidya, T. Ahmad, *B Mater Sci* 31 (2008) 415.
38. J. Rockenberger, E.C. Scher, A.P. Alivisatos, *J American Chem Soc* 121 (1999) 11595.
39. C. Wang, J Li, G Mele, G-M Yang, F-X Zhang, L Palmisano, G Vasapollo, *App Catal B* 76 (2007) 218.
40. G.M. Neelgund, A. Oki, Z. Luo, *J Colloid Interf Sci* 430 (2014) 257.
41. G. Mattioli, F. Filippone, P. Alippi, P. Giannozzi, A.A. Bonapasta, *J Mater Chem* 22 (2012) 440. b) M. Izaki, R. Chizaki, T. Saito, K. Murata, J. Sasano, T. Shinagawa, *ACS Appl Mater Interf* 5 (2013) 9386. c) G.D. Sharma, R. Kumar, S. K. Sharma, M.S. Roy, *Sol Energy Mater Sol C* 90 (2006) 933.
42. P. Gregory. *High-Technology Applications of Organic Colourant*, Plenum Press, New York (1991) pp. 7-273.
43. N.B. McKeown. *Phthalocyanine Materials: Synthesis, Structure and Function*. Chemistry of Solid State Materials, Cambridge University Press, New York (1998) pp. 32-149.
44. J.A. de Saja, M.L. Rodríguez-Méndez, *Adv Colloid Interfac* 116 (2005) 1.
45. K. Morishige, S. Tomoyasu, G. Iwani, *Langmuir* 13 (1997) 5184.

BIBLIOGRAPHY

46. T. Nyokong, in J.H. Zagal, F. Bedioui F, Dodelet JP (Eds.), *N4-Macrocyclic Metal Complexes*, Springer, New York, (2006).
47. E.A. Lukyanets, *J Porphyr Phthalocya* 3 (1999) 424.
48. C.M. Whitacre, D.K. Feyes, T. Satoh, J. Grossmann, J.W. Mulvihill, H. Mukhtar, N.L. Oleinick, *Clin Cancer Res* 6 (2000) 2021.
49. H. Kolarova, K. Tomankova, R. Bajgar, P. Kolar, R. Kubinek, *Ultrasound Med Bio* 35 (2009) 1397.
50. M. Tian, T. Wada, H. Kimura-Sudab, H. Sasabea, *J Mater Chem* 7 (1997) 861.
51. R.C. Baker, D.P. Wilkinson, J.J. Zhang, *ECS Trans* 16 (2009) 43.
52. M. K. Nazeeruddin, R. Humphry-Baker, M. Grätzel, B. A. Murrer, *Chem Commun* (1998) 719.
53. R.P. Linstead, *J Chem Soc* (1934) 1016.
54. T. Nyokong, *Coord Chem Rev* 251 (2007) 1707.
55. V.N. Nemykin, E.A. Lukyanets, *ARKIVOC* 1 (2010) 136.
56. W. Chidawanyika, T. Nyokong, *J Photochem Photobiol A: Chem* 202 (2009) 99.
57. P. Yiru, H. Fenghua, L. Zhipeng, C. Naisheng, H. Jinling, *Inorg Chem Commun* 7 (2004) 967.

BIBLIOGRAPHY

58. J.G Young, W. Onyebuagu, *J Org Chem* 55 (1990) 681.
59. C.C. Leznoff, M. Hu, C.R. McArthur, Y. Qin, J.E. van Lier, *Can J Chem* 72 (1994) 1990.
60. V.M. Vlasov, *Russ Chem Rev* 72 (2003) 681.
61. G. Schmid, M. Sommerauer, M. Hanack, *Angew. Chem Int Ed Engl* 32 (1993) 1422.
62. M. Sommerauer, C. Rager, M. Hanack, *J Am Chem Soc* 118 (1996) 10085.
63. M. Hanack, D. Meng, A. Beck, M. Sommerauer, L.R. Subramanian, *J Chem Soc Chem Commun* (1993) 58.
64. M. Sangermano, G. Malucelli, A. Priola, S. Lengvinaite, J. Simokaitiene, J.V. Grazulevicius, *Eur Polymer J* 41 (2005) 475.
65. W.P. Hu, Y.K. Chen, C.C. Liao, H.S. Yu, Y.M. Tsai, S.M. Huang, F.Y. Tsai, H.C. Shen, L.S. Chang, J.J. Wang, *Bioorg Med Chem* 18 (2010) 6197.
66. S.J. Lee, K.H. Min, H.J. Lee, A.N. Koo, H.P. Rim, B.J. Jeon, S.Y. Jeong, J.S. Heo, S.C. Lee, *Biomacromolecule* 12 (2011) 1224.
67. A. Feofanov, A. Grichine, T. Karmakova, N. Kazachkina, E. Pecherskih, R. Yakubovskaya, E. Luk'yanets, V. Derkacheva, M. Egret-Charlier, P. Vigny, *J Photochem Photobiol* 75 (2002) 527.

BIBLIOGRAPHY

68. T. Karmakova, A. Feofanov , A. Nazarova, A. Grichine, R. Yakubovskaya, E. Luk'yanets, J.-C. Maurizot, P. Vigny, *J Photochem Photobiol B: Biol* 75 (2004) 81.
69. A.B.P. Lever, *Adv Inorg Radiochem* 7 (1965) 27.
70. M.J. Stillman, T. Nyokong, In *Phthalocyanines: Properties and Applications*, eds. C.C. Leznoff, A.B.P. Lever, VCH publishers, New York, Vol. 1 (1989) 133.
71. M. Gouterman, in *The Porphyrins, Part A. Physical Chemistry*, D. Dolphin (Ed.), Academic Press, New York, Vol. 3 (1978) 1.
72. A.R. Monahan, J.A. Brado, F.A. DeLuca, *J Phys Chem* 76 (1972) 446.
73. W.A. Nevin, W. Liu, S. Greenberg, M.R. Hempstead, S.M. Maruccio, M.M. Melnik, C.C. Leznoff, A.B.P. Lever, *Inorg Chem* 26 (1987) 291.
74. E. Schnabel, H. Nöther, H. Kuhn, in *Chemistry of Natural and Synthetic Colouring Matters*, T.S. Gore, B.S. Joshi, S.V. Sunthakar, B.D. Tilak (Eds.), Academic Press, New York, 1962, 561-572.
75. A. Skorobogaty, T.D. Smith, G. Dougherty, J.R. Pilbrow, *J Chem Soc Dalton Trans* (1985) 651.
76. M. Kasha, *Radiat. Res.*, 1960, 20, 55; Z. Gaysna, N. Kobayashi, M.J. Stillman, *J Chem Soc Dalton Trans* (1989) 2397.
77. A. Jablonski, *Z Phys* 94 (1935) 38.

BIBLIOGRAPHY

78. P.W. Atkins, in *Physical Chemistry*, P.W. Atkins (Ed.), Oxford University Press, Oxford, 6th Edition, 1998, Chapter 17.
79. K. Ishii, N. Kobayashi, in *The Porphyrin Handbook*, K.M. Kadish, K.M. Smith, R. Guilard, (Eds.)Elsevier Science, New York, Vol 16, 2003, Chapter 102.
80. D. R. Arnold, N. C. Baird, J. R. Bolton, J. C. D. Brand, P. W. M. Jacobs, P. de Mayo, W. R. Ware, *Photochemistry: An Introduction*, Academic Press, New York, 1974
81. A. Harriman, M.C. Richoux, *J Chem Soc Faraday Trans II* 76 (1980) 1618.
82. A. Ogunsipe, J.Y. Chen, T. Nyokong, *New J Chem* 28 (2004) 822.
83. S. Fery-Forgues, D. Lavabre, *J Chem Ed* 76 (1999) 1260
84. J. Turro, in *Modern Molecular Photochemistry*, The Benjamin/Cummings Publishing Co Inc, New York (1978)
85. T. H. Tran-Thi, C. Desforge, C. Thiec, *J Phys Chem* 93 (1989) 1226
86. M. Niedre, M.S. Patteerson, B.C. Wilson, *J Photochem Photobiol* 75 (2003) 382.
87. M.S. Patterson, S.J. Madsen, R. Wilson, *J Photochem Photobiol B: Bio* 5 (1990) 69.
88. N. Kuznetsova, N. Gretsova, E. Kalmykova, E. Makarova, S. Dashkevich, V. Negrimovskii, O. Kaliya, E. Lukyanets, *Rus. J. Gen. Chem.* 70 (2000) 133

BIBLIOGRAPHY

89. C.S. Foote, in Singlet Oxygen, Wasserman H.H., Murray R.W. (Eds). Academic Press, New York, San Francisco, London, 1979: 139-171
90. Y. Cheng, A.C. Samia, J. Li, M.E. Kenney, A. Resnick, C. Burda, *Langmuir* 26 (2010) 2248.
91. N. Nombona, K. Maduray, E. Antunes, A. Karsten, T. Nyokong, *J Photochem Photobiol B: Biol* 107 (2012) 35
92. M.E. Wieder, D.C. Hone, M.J. Cook, M.M. Handsley, J. Gavrilovic, D.A. Russell, *Photochem Photobiol Sci* 10 (2011) 822.
93. M. Camerin, M. Magaraggia, M. Soncin, G. Jori, M. Moreno, I. Chambrier, M.J. Cook, D.A. Russell, *European J Cancer* 46 (2010) 1910.
94. N. Masilela, E. Antunes, T. Nyokong, *J Porphyr Phthalocya* 17 (2013) 417.
95. S. Moeno, E. Antunes, T. Nyokong, *J Photochem Photobiol A: Chem* 222 (2011) 343.
96. E. Vargas, R. Vargas, O. Núñez, *Appl Catal B: Environ* 8 (2014) 156.
97. A. Ebrahimian, M.A. Zanjanchi, H. Noei, M. Arvand, Y. Wang, *J Environ Chem Eng* 2 (2014) 484.
98. K. Vignesh, M. Rajarajan, A. Suganthi, *Mater Sci Semicon Proc* 23 (2014) 98
99. K.T. Ranjit, I. Willner, S. Bossmann, A. Braun, *J Phys Chem B* 102 (1998) 9397.

BIBLIOGRAPHY

100. P. Modisha, T. Nyokong, *Inte J Nanosci* 12 (2013) 1-10
101. L. Xia , X. Kong , X. Liu, L Tu , Y. Zhang , Y. Chang , K. Liu , D. Shen, H. Zhao , H. Zhang, *Biomaterials* 35 (2014) 4146.
102. B. Zhao, J.-J. Yin, P.J. Bilski, C.F. Chignell. J.E. Roberts, Y.-Y. He, *Toxicol Appl Pharm* 241 (2009) 163.
103. A. Fashina, E. Antunes, T. Nyokong, *J Pophyri Phthalocya* 18 (2014) 401.
104. N.B. Sultimova, P.P. Levin, A.V. Lobanov, A.M. Muzafarov, *High Energy Chem* 47 (2013) 98.
105. X. Ma, S. Sreejith, Y. Zhao, *ACS Appl Mater Interf* 5 (2013) 12860.
106. R.R. Salinas-Guzmán, J.L. Guzmán-Mar, L. Hinojosa-Reyes, J. M. Peralta-Hernandez, A. Hernandez-Ramirez, *J Sol-Gel Sci Technol* 54 (2010) 1,
107. A. Yarahmadi, S. Sharifnia, *Dyes and Pigments* 107 (2014) 140.
108. O. Raab, *Z Biol* 39 (1900) 524.
109. F. Meyer-Betz, *Dtsch Arch Klin Med* 112 (1913) 476.
110. A. Staicu, APascu, A Nuta, A. Sorescu, V. Raditoiu, M.L. Pascu, *Rom Rep Phy* 65 (2013)1032.

BIBLIOGRAPHY

111. R.R. Allison, G.H. Downie, R. Cuenca, X.-H. Hu, C.J.H. Childs, C.H. Sibata, *Photodiagn Photody Ther* 1 (2004) 27.
112. C.M. Allen, W.M. Sharman, J.E. van Lier, *J Porphyr Phthalocya* 5 (2001) 161.
113. P.S Low, W.A. Henne, D.D. Doorneweerd, *Acc Chem Res* 41 (2008) 120.
114. L.B. Josefsen, R.W. Boyle, *Brit J Pharm* 154 (2008) 1.
115. C.P. Leamon, J.A. Reddy, *Adv Drug Deliv Rev* 56 (2004) 1127.
116. T.M. Allen, *Nature Rev* 2 (2002) 750.
117. S. Wang, P.S. Low, *J Controlled Release* 53 (1998) 39.
118. Y. Lu, P.S Low, *Adv Drug Deliv Rev* 54 (2002) 675.
119. R.I Pinhassi, Y.G. Assaraf, S. Farber, M. Stark, D. Ickowicz, S. Drori, A.J. Domb, Y.D. Linvey, *Biomacromolecule* 11 (2010) 294.
120. A. Gabizon, A.T. Horowitz, D. Goren, D. Tzemach, F. M. Shavit, M.M. Qazen, S. Zalipsky, *Bioconjugate Chem* 10 (1999) 289.
121. C.P. Leamon, J.A. Reddy, *Adv Drug Deliv Rev* 56 (2004) 1127.
122. M.M. Qualls, D.H. Thompson, *Int J Cancer* 93 (2001) 384.
123. N. Nombona, T. Nyokong, *Inorg Chimica Acta* 392 (2012) 380.

BIBLIOGRAPHY

124. F. Danhier, O. Feron, V. Préat, *J Controlled Release* 148 (2010) 135.
125. V.P. Torchilin, *European Pharmaceutical Sciences* 11 Suppl 2 (2000) S81.
126. A.K. Iyer, G. Khaled, J. Fang, H. Maeda, *Drug Discovery Today* 11 (2006) 812.
127. K. Greish, *J Drug Targeting* 15 (2007) 457.
128. J. Fang, H. Nakamura, H. Maeda, *Adv Drug Deliv Rev* 63 (2011) 136.
129. H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, *J Controlled Release* 65 (2000) 271.
130. H. Maeda, *Bioconjugate Chem* 21 (2010) 797.
131. J. Khandare, T. Minko, *Prog Polym Sci* 31 (2006) 359.
132. H. Maeda, G.Y. Bharate, J. Daruwalla, *European J Pharmaceutics Biopharmaceutics* 71 (2009) 409.
133. J. Chen, Z. Chen, Y. Zheng, S. Zhou, J. Wang, N. Chen, J. Huang, F. Yan, M. Huang, *J Porphyr Phthalocya* 15 (2011) 293.
134. N. Nombona, E. Antunes, W. Chidawanyika, P. Kleyi, Z. Tshentu, T. Nyokong, *J Photochem Photobiol A: Chem* 233 (2012) 24.
135. R. de-C.S. Luz, F.S. Damos, A.A. Tanaka, L.T. Kubota, *Sensor Actuator B* 114 (2006) 1019.

BIBLIOGRAPHY

136. M. Hejmadi, Introduction to Cancer Biology, http://web.mef.hr/web/images/pdf/int_canc_b.pdf, Access date: 28/09/2014.
137. R. Marks, Clin Exp Dermatol 25 (2000) 459.
138. G.-E. Costin, V.J. Hearing, *FASEB J* 21 (2007) 971.
139. D.J. O'Shannessy, E.B Somers, J. Maltzman, R. Smale, Y.-S. Fu, Springer Plus 22 (2012) 1.
140. M.S. Jhaveri, A.S. Rait, K.-N. Chung, J.B. Trepel, E.H. Chang, Mol Cancer Therapy 3 (2004) 1505.
141. M. Campo, D. Gabriel, P. Kucera, R. Gurny, N. Lange, Photochem Photobiol 83 (2007) 958.
142. D. Gabriel, M. Campo, R.Gunny, N. Lange, Bioconjugate Chem 18 (2007) 1070.
143. D. Gabriel, M.-F. Zualaga, M.-N. Martinez, M. Campo, N. Lange, J Drug Deliv Sci Technol 19 (2009) 15.
144. R. Andreozzi, V. Caprio, A. Insola, R. Marotta, Catal Today 53 (1999) 51.
145. M. Pera-Titus, V. García-Molina, M.A. Baños, J. Giménez, S. Esplugas, Appl Catal B 47 (2004) 219.
146. P.Y. Chan, M.G El-Din, J.R Bolton, Water Res 46 (2012) 5672.

BIBLIOGRAPHY

147. R. Munter, *Proc Estonian Acad Sci Chem* 50 (2001) 59.
148. T. Robinson, G. McMullan, R. Marchanr, P. Nigam, *Bioresour Technol* 77 (2001) 247.
149. E. Neyens, J Baeyens, *J Hazard Mater* 98 (2003) 33.
150. E. Forgacs, T. Cserhati, G. Oros, *Environ Inte* 30 (2004) 953.
151. R. Zugle, S Khene, E Antunes, T Nyokong, *Polyhedron* 33 (2012) 74.
152. R. Zugle, T Nyokong, *J App Polym Sci* 128 (2013) 1131.
153. S. Tombe, E Antunes, T Nyokong, *J Mol Catal A: Chem* 371 (2013) 125.
154. S. Wang, Y. Fang, Y. Yang, J. Liu, A. Deng, X. Zhao, Y. Huang, *Chinese Sci Bull* 56 (2011) 969.
155. H. Golmojdeh, M.A. Zanjanchi, M. Arvad, *Photochem Photobiol* 89 (2013) 1029.
156. C.M. Whitacre, D.K. Feyes, T. Satoh, J. Grossmann, J.W. Mulvihill, H. Mukhtar, N.L. Oleinick, *Clin Cancer Res* 6 (2000) 2021.
157. D. Jancula , L. Bláhová, M. Karásková , B. Marsálek, *Water Sci Technol* 62 (2010) 273.
158. R. Zugle, T. Nyokong, *J Mol Catal A: Chem* 358 (2012) 49.

BIBLIOGRAPHY

159. P. Modisha, T. Nyokong, E. Antunes, *J Mol Catal A: Chem* 380 (2013) 131.
160. Z. Guo, B. Chen, J. Mu, M. Zhang, P. Zhang, Z. Zhang, J. Wang, X. Zhang, Y. Sun, C. Shao, Y. Liu, *J Hazard Mater* 219 (2012) 156.
161. S.-L. Cheng, X.-J. Huang, Z.-K. Xu, *Cellulose* 18 (2011) 1295.
162. C. Shen, S. Song, L. Zang, X. Kang, Y. Wen, W. Liu, L. Fu, *J Hazard Mater* 177 (2010) 560.
163. A.B. Sorokin, F.Q.R. Valentin, S. Mangematin, *Appl Catal A: Gen* 309 (2006) 162.
164. A. K. Amanbaeva, *Candidates Dissertation*, Karaganda University, 2000
165. S. Ramakrishna, K. Fujihara, W.-E. Teo, T.-C. Lim, Z. Ma, *Intoroduction to electrospinning and nanofibers*, World Scientific Publishing Co. Pte. Ltd, Singapore, 2005.
166. T. Hobbs, A.J. Lesser, *Polymer* 41 (2000) 6223.
167. Y. Wang, M. Zheng, H. Lu, S. Feng, G. Ji, J. Cao, *Nanoscale Res Lett* 5 (2010) 913.
168. C.C. Lee, C. Grenier, E.W. Meijer, A.P.H.J. Schenning, *Chem Soc Rev* 38 (2009) 671.
169. J. Zhao, W. Han, H. Chen, M. Tu, R. Zeng, Y. Shi, Z. Cha, C. Zhou, *Carbohydr Polym* 83 (2011) 1541.

BIBLIOGRAPHY

170. Z.-M. Huang, Y.-Z. Zhang, M. Kotaki, S. Ramakrishna, *Composite Sci Technol* 63 (2003) 2223.
171. J. Doshi, D.H. Reneker, *J Electrostat* 35 (1995) 151.
172. G.C. Rutledge, S.V. Fridrikh, *Adv Drug Deliv Rev* 59 (2007) 1384.
173. D. Li, Y. Xia, *Adv Mater* 16 (2004) 1151.
174. R. Herrero-Vanrell, M.F. Refojo, *Adv Drug Deliv Rev* 52 (2001) 5.
175. S. Freiberg, X.X. Zhu, *Int J Pharm* 282 (2004) 1.
176. H. Kawaguchi, *Prog Polym Sci* 25 (2000) 1171.
177. M.N.V. Ravi Kumar, *Reac Funct Polym* 46 (2000) 1.
178. M. Rinaudo, In *Bio-based Plastics*, eds. S. Kabacci, John Wiley & Sons Ltd, Chichester, UK (2014) 63-87.
179. K.V. Harish Prashanth, R.N. Tharanathan, *Trend Food Sci Technol* 18 (2007) 117.
180. A. Bhatnagar, M. Sillanpää, *Adv Colloid Interf Sci* 152 (2009) 26.
181. G. Crini, P.-M. Badot, *Prog Polym Sci* 33 (2008) 399.
182. W.S. Wan Ngah, L.C. Teong, M.A.K.M. Hanafiah, *Carbohydr Polym* 83 (2011) 1446.

BIBLIOGRAPHY

183. E. Guibal, *Sep Purif Technol* 38 (2004) 43.
184. H. Momenzadeh, A.R. Tehrani-Bagha, A. Khosravi, K. Gharanjig, K. Holmberg, *Desalination* 271 (2011) 225.
185. H. Ennajih, R. Bouhfid, E.M. Essassi, M. Bousmina, A. El Kadib, *Micropor Mesopor Mater* 152 (2012) 208.
186. H. Homayoni, S.A.H.Ravandi, M. Valizadeh, *Carbohydr Polym* 77 (2009) 656.
187. V.R. Sinha, A.K. Singla, S. Wadhawan, R. Kaushik, R. Kumria, K. Bansal, S. Dhawan, *Int J Pharm* 274 (2004) 1.
188. D. Lutic, C. Coromelci-Pastravanu, I. Cretescu, I. Poullos, C.-D. Stan, *Int J Photoenergy* 2012 (2012) 1.
189. R. Nagaraja, N. Kottam, C.R. Girija, B.M. Nagabhushana, *Powder Technol* 215 (2012) 91.
190. D. Kornbrust, T. Barfknecht, *Environ Mutagen* 7 (1985) 101.
191. H.B. Mansour, D. Corroler, D. Barillier, K. Ghedira, L. Chekir, R. Mosrati, *Food Chem Toxicol* 45 (2007) 1670.
192. I.K. Konstantinou, T.A. Albanis, *App Catal B: Environ* 49 (2004) 1.
193. F. Kiriakidou, D.I. Kondarides, X.E. Verykio, *Catal Today* 54 (1999) 119.

BIBLIOGRAPHY

194. E. Guivarch, S.T.C. Lahitte, M.A. Oturan, *Environ Chem Lett* 1 (2003) 38.
195. E. Forgacs, T. Cserhátia, G. Orosb, *Environment Int* 30 (2004) 953.
196. B.N Achar, G.M. Fohlen, J.A. Parker, J. Keshavayya, *Polyhedron* 6 (1987) 1463.
197. Y. Arslanoglu, M. Idowu, T. Nyokong, *Spectrochim Acta A* 95 (2012) 407.
198. J.R Lakowicz in: *Principles of Fluorescence Spectroscopy*, 2nd ed., Kluwer Academic/Plenum Publishers, New York, 1999
199. C.S. Enwemeka, *Photomed Laser Surg* 27 (2009) 387.
200. E.T. Saka, C. Gol, M. Durmus, H. Kantekin, Z. Biyikoglu, *J Photochem Photobiol A: Chem* 241 (2012) 67
201. D.W. Bahnemann, C. Kormann, M.R. Hoffmann, *J Phys Chem* 91 (1987) 3789.
202. A.M. Shaheer, K.M. Alam, J.M. Seok, Y.O Bong, *Electrochim Acta* 53 (2008) 7869.
203. D.C. Hone, P.I. Walker, R. Evans-Gowing, S. FitzGerald, A. Beeby, I. Chambrier, M.J. Cook, D.A. Russell, *Langmuir* 18 (2002) 2985.
204. S. Tang, C. Shao, Y. Liu, S. Li, R. Mu. *J Phys Chem Solids* 68 (2007) 2337.
205. M. Jabli, M.H.V. Baouab, N. Sintès-Zydowicz, B.B. Hassine, *J App Poly Sci* 123 (2011) 3412.

BIBLIOGRAPHY

206. S.M. Marcuccio, P.I. Svirskaya, S. Greenberg, A.B.P. Lever, C.C. Leznoff, K.B. Tomer, *Can J Chem* 63 (1985) 3057.
207. A.Y. Tolbin, V.E. Pushkarev, G.F. Nikitin, L.G. Tomilova, *Tetrahedron Lett.* 69 (2009) 4848–4850.
208. R. Schneider, F. Schmitt, C. Frochot, Y. Fort, N. Lourette, Francois Guillemain, J. Muller and M. Barberi-Heyob, *Bioorg. Med Chem* 13 (2005) 2799.
209. A. Ogunsipe, D. Maree, T. Nyokong, *J Mol Struct* 650 (2003) 131.
210. T. Fukuda, S. Homma, N. Kobayashi, *Chem Eur J* 11 (2005) 5205.
211. A. Erdoğan, A. Ogunsipe, T. Nyokong, *J Photochem Photobiol A: Chem* 205 (2009) 12.
212. K. Kasuga, N. Matsuura, K. Inoue, M. Handa, T. Sugimori, K. Isa, M. Nakata, *Chem Lett* 3 (2002) 352.
213. M.K. Off, A.E. Steindal, A.C. Porojnicu, A. Juzeniene, A. Vorobey, A. Johnsson, J. Moan, *J Photochem Photobiol B: Biol* 80 (2005) 47.
214. B.N. Achar, K.S. Lokesh. *J Organomet Chem* 689 (2004) 2601.
215. D. Sun, H.J. Sue, N. Miyatake, *J Phys Chem C* 112 (2008) 16002.
216. J.D. Lamba, A.Y. Nazarenko, J. Uenishi, H. Tsukube, *Analytica Chimica Acta* 373 (1998) 167.

BIBLIOGRAPHY

- 217. F. Angela, D. de Namor, O.E. Piro, L.E.P Salazar, A.F. Aguilar-Cornejo, N. Al-Rawi, E.E. Castellanoc, F.J.S Velardea, *J Chem Soc Faraday Trans* 94 (1998) 3097-3104.
- 218. S. He, J. Yao, P. Jiang, D. Shi, H. Zhang, S. Xie, S. Pang, H. Gao, *Langmuir* 17 (2001) 1571.
- 219. X.-F. Zhang, X. Li, L. Niu, L. Sun, L. Liu, J. *Fluorescence* 19 (2009) 947.
- 220. T.T.T Mai, P.T. Ha, H.N. Pham, T.T.H. Le, H.L. Pham, T.B.H Phan, D.L. Tran, X.P. Nguyen, *Adv Nat Sci: Nanosci Nanotechnol* 3 (2012) 1.
- 221. K.M. Gregorio-Jauregui, M.G. Pineda, J.E. Rivera-Salinas, G.H. Saade, J.L. Martinez, A. Ilyina, R.G. López, *J Nanomat* 2012 (2012) 1.
- 222. W. Freyer, S. Mueller, K. Teuchner, *J Photochem Photobiol A: Chem* 163 (2004) 231.
- 223. A. Grofcsik, P. Baranyai, I. Bitter, V. Csokai, M. Kubinyi, K. Szegletes, J. Tatai, T. Vidoczy, *J Mol Struct* 704 (2004) 11.
- 224. T. Nyokong, E. Antunes, in: *The Handbook of Porphyrin Science*. Eds. K.M. Kadish, K.M. Smith, R. Guillard, Academic Press: New York, Vol. 7, World Scientific, Singapore 2010; chapter 34: 247-349
- 225. J.-H. Ha, G.Y. Jung, M.-S. Kim, Y.H. Lee, K. Shin, Y.-R. Kim, *Bull Korean Chem Soc* 22 (2001) 63.
- 226. N. Nombona, W. Chidawanyika, T. Nyokong, *Polyhedron* 30 (2011) 654.

BIBLIOGRAPHY

227. X.-F. Zhang, Y. Di, F. Zhang, *J Photochem Photobiol A: Chem* 203 (2009) 216.
228. M. G. Debacker, O. Deleplanque, B. Van Vlieberge, F.X. Sauvage, *Laser Chem* 8 (1988) 1.
229. E. Antunes, N. Rapulenyane, M. Ledwaba, C. Litwinski, W. Chidawanyika, T. Nyokong, *Inorg Chem Commun* 29 (2013) 60.
230. A. Kotiaho, R. Lahtinen, A. Efimov, H.-K Metsberg, E. Sariola, H. Lehtivuori, N.V. Tkachenko, H. Lemmetyinen, *J Phys Chem C* 114 (2010) 162.
231. J.R. Darwent, P. Douglas, A. Harriman, G. Potter, M.C. Richoux, *Coord Chem Rev* 44 (1982) 83-.
232. F. Chen, J Zhao, H Hidaka, *Int J Photoenergy* 5 (2003) 209.
233. S.-M. Lam, J.-C. Sin, A.Z. Abdullah, A.R. Mohamed, *Desalin Water Treat* 41 (2012) 131.
234. J.Z. Kong, A.D. Li, X.Y. Li, H.F. Zhai, W.Q. Zhang, Y.P. Gong, H. Li, D. Wu, *J Solid State Chem* 183 (2010) 1359.
235. N. Sobana, M. Swaminathan, *Sol Energy Mater Sol Cells* 91 (2007) 727.
236. H. Wang, C. Xie, W. Zhang, S. Cai, Z. Yang, Y. Gui, *J Hazard Mater* 141 (2007) 645.
237. M.A. Fox, M.T. Dulay, *Chem Rev* 93 (1993) 341.

BIBLIOGRAPHY

238. B. Cabır, B. Avar, M. Gülcan, A. Kayraldiz, M. Kurtoglu, Turk J Chem 37 (2013) 422–438.
239. S.K. Cushing, J. Li, F. Meng, T.R. Senty, S. Suri, M. Zhi, M. Li, A.D. Bristow, N. Wu, J Am Chem Soc 134 (2012) 15033.
240. H. Gao, C. Liu, H.E. Jeong, P. Yang, ACS Nano 6 (2012) 234.
241. L. Kuai, B. Geng, X. Chen, Y. Zhao, Y. Luo, Langmuir 26 (2010) 18723.
242. P. Wang, B. Huang, Z. Lou, X. Zhang, X. Qin, Y. Dai, Z. Zheng, X. Wang, Chem Eur J 16 (2010) 538.
243. W. Hou, S.B. Cronin, Adv Funct Mater 23 (2013) 1612.
244. L. Shao, B. Hu, P. Dong, W. Ji, C. Qi, J Porphyr Phthalocya 14 (2010) 993.
245. R. Zugle, T. Nyokong, J Mol Cat A: 366 (2013) 247.
246. E. Borenfreund, J.A. Puerne, Method Cell Sci 9 (1985) 7.
247. J.A. Werner; A.A. Dunne; B.J. Folz; B.M. Lippert, J Moffitt Cancer Centre 9 (2002) 379.
248. L. Frigo, J.S.S. Luppi, G.M. Favero, D.A Maria, S.C. Penna, J.M. Bjordal, R.J. Bensadoun, R.A.B. Lopes-Martins, , BMC Cancer 404 (2009)1.

BIBLIOGRAPHY

- 249. C.M.N Yow, N.K. Mak, A.W.N. Leung, Z. Huang, Photodiagn Photodyn Ther 6 (2009) 122.
- 250. L. Zhou, X. Ge, J. Liu, J. Zhou, , S. Wei, , F. Li, J. Shen, Bioorg Medicinal Chem Lett 23 (2013) 5317.
- 251. M.P. Siqueira-Moura, F.L Primo, E.M. Espreafico, A.C. Tedesco, Mater Sci Eng C: Mater Biol Appl 33 (2013) 1744.
- 252. N.V. Kudinova, T.T. Berezov, Biochem (Moscow) Supp Series B: Biomed Chem 4 (2010) 95.
- 253. T. Patrice in Photodynamic therapy (Eds. D.P. Häder, G. Jori) European Society for Photobiology, Cambridge, 2003, 4-8
- 254. I Seotsanyana-Mokhosi, T. Kresfelder, H. Abrahamse, T. Nyokong, J Photochem Photobiol B: Biol 83 (2006) 55.
- 255. A.P. Castano, T.N. Demidova, M.R. Hamblin, Photodiag Photodynam Ther 2 (2005) 1.
- 256. D. Atilla, N. Saydan, M. Durmus, A.G. Gurek, T. Khan, A. Ruck, H. Walt, T. Nyokong, V. Ahsen, J Photochem Photobiol A: Chem 186 (2007) 298.