

**Magneto-Fluorescent Carbon Coated Superparamagnetic Iron Oxide
Nanoarchitectures (SPIONs) for Multimodal Imaging and Cancer Theranostics**

A thesis submitted for the award of the degree of

Doctor of Philosophy

By

Ashish Tiwari



School of Engineering
Indian Institute of Technology, Mandi
Himachal Pradesh-175005, India
November 2020

Preface

The present thesis entitled “Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for multimodal imaging and cancer theranostics” is submitted in candidacy for the award of a PhD degree from Indian Institute of Technology (IIT) Mandi. The work presented in this thesis was carried out during the period of August 2015 to June 2020 at School of Engineering, IIT Mandi under the guidance of Dr. Jaspreet Kaur Randhawa.

The research work was fully funded by Ministry of Human Resource Development (MHRD), Government of India. The central hypothesis of the work was to design novel magneto-fluorescent nanoarchitectures for multimodal imaging in cancer theranostics for biomedical applications. The work is dully supported with peer reviewed published articles and patent.

IIT Mandi, established in 2009, has rapidly risen among the premier institutes in India. It is located in the Kamand valley on the banks of Uhl, a tributary of the river Beas. Kamand is approximately 14 kms from Mandi town and has an average elevation of 1044 meters from sea level. There is great variation in the climatic conditions of Himachal due to extreme variation in elevation. The climate varies from hot and sub humid tropical in the southern tracts to cold, alpine and glacial in the northern and eastern mountain ranges with more elevation.

Unusually, it is worth mentioning the Global Pandemic of Coronavirus (COVID-19) declared on March 11, 2020 by WHO, which made this journey little sinister and ominous particularly during inclusive Lockdown.



Thesis Certificate

This is to certify that the thesis entitled “**Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for multimodal imaging and cancer theranostics**” submitted by **Mr. Ashish Tiwari** to the Indian Institute of Technology, Mandi for the award of the degree of **Doctor of Philosophy (PhD)** is an original record of research work carried out by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

Date:

Dr. Jaspreet Kaur Randhawa

Place: Mandi

Assistant Professor
School of Engineering
Indian Institute of Technology, Mandi
Himachal Pradesh -175001, India



Declaration by the Research Scholar

This is to certify that the thesis entitled “**Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for multimodal imaging and cancer theranostics**”, submitted by me to the Indian Institute of Technology Mandi for the award of the degree of **Doctor of Philosophy (PhD)** is an original record of research work carried out by me under the supervision of **Dr. Jaspreet Kaur Randhawa**. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

Date:

Place: Mandi

Ashish Tiwari

PhD Scholar

School of Engineering

Indian Institute of Technology, Mandi

Himachal Pradesh -175005, India

Acknowledgement

First and foremost respect and thank to the God, the Almighty, for his showers of blessings throughout the journey to complete the research work fruitfully. I also convey sincere respect to my family members for their love, affection and unconditional support throughout the expedition.

Formally, I am feeling fortunate to take this opportunity to thank one and all those helped me throughout this journey. I would like to express sincere gratitude to my supervisor Dr. Jaspreet Kaur Randhawa. In the last several years, Dr. Randhawa guided me continuously in my research and scientific improvement as an admirable friend and always encouraged me all the way possible. We thoroughly worked together and discussed all the scientific problems and challenges amide the research expedition. I extend my deep thanks and gratitude to my doctoral committee members Dr. Viswanath Balakrishnan, Dr. Vishal Singh Chauhan, Dr. Rajeev Kumar, Dr. Rajanish Giri and Dr. Swati Sharma for their valuable advice and guidance to complete the work. I also thank to my collaborators Dr. Orit Shefi, Dr. Chayan Kanti Nandi, Dr. Anup Singh, Dr. Neha Garg, Dr. Rashi Mathur, Dr. Grald Dreager and Dr. Ankur Kaul for their guidance, scientific contributions and endless support throughout the course to complete the research work productively. Indeed, it was a great privilege and stature working under their guidance and knowledge. I also convey my sincere thanks to Dr. Ramasamy Sakthivel for his kind support during my initial retro of research in engineering. I also convey my sincere thanks to Prof. S. K. Nayak for his guidance and support amid M.Tech dissertation. I wish to thank my research collaborator colleagues Dr. Raj Kumar, Dr. Navneet Verma, Dr. Ayan Debnath, Dr. Sibel Turkkan and Mr. Ashutosh Singh for their scientific contributions making it a great success with high throughput valuable efforts.

Additionally, I would like to thank my colleagues and lab mates who have sincerely contributed knowingly and unknowingly throughout this memorable journey. I wish to thank some of my graduated students and trainee/interns students for their valuable help and scientific contribution during the delighted expedition of my research. I also thank to some of my school/college friends for their love and affection to achieve this feat. I also acknowledge IIT Mandi, AMRC and BioX IIT Mandi, respective authorities and colleagues for their kind and valuable support to complete this memorable journey. My sincere gratitude to MHRD, Govt. of India for the award of fellowship to pursue research with accomplished endeavor and strength.

Unusually, it is worth mentioning the Global Pandemic of Coronavirus (COVID-19) declared on March 11, 2020 by WHO, which made this journey little sinister and ominous particularly during inclusive Lockdown.

Last but not the least, I thank to one and all, who have been associated throughout my journey to complete this dissertation, for their love, affection and unconditional livelihood to make this dream come true.

Abstract

Cancer has been, and still remains, one of the most chronic disease to treat. As a result of severe adverse effects experienced from current cancer treatment and clinical trial studies, there has been a consistent growing interest in the development of an efficient cancer theranostics system that can effectively cure the cancer, but render healthy tissue unharmed. A prior objective of the present thesis was to develop such cancer nanotheranostics systems and evaluate their therapeutic efficacy in real time cancer theranostics with full proof of concept strategies.

The central hypothesis of this thesis was to enable multimodal imaging ability in magnetic nanoparticles by associating fluorescence in to their structures and insitu tuning of the magneto-fluorescent properties. Multifunctional magneto-fluorescent nanoarchitectures were developed in an easy and facile single step synthesis method avoiding multistep process and any kind of post synthesis modifications. A full proof of the property tuned synthesis protocol is described and proven through the characterization results. Advanced with the development of three different magneto-fluorescent nanoarchitectures, we evaluated their potential in MR imaging, fluorescence imaging, single particle imaging and tracking respectively. In addition, we also explored the equivalent value for the use of magneto-fluorescent nanoarchitectures in stimuli responsive drug delivery, magnetic hyperthermia, neuroengineering, protein sensing and magnetic field induced fluorescence engineering applications. This thesis successfully achieved all the above biomedical applications and significantly addresses the challenges as stated above and stand potentially in achieving the high throughput results in real time cancer theranostics.

In summary, magneto-fluorescent carbon coated superparamagnetic iron oxide (SPIONs) nanoarchitectures especially designed to practically confronting property oriented applications, persistent with physio-chemical and biological experimental studies, have been established as a promising proof of concept for real time multimodal imaging, neuroengineering and cancer theranostics in biomedical applications.

List of Patent and Publications

Patent

1. **Ashish Tiwari** et al. Single Step Synthesis of Multimodal Magneto-Fluorescent Core-Shell Superparamagnetic Iron Oxide Nanoparticles and Fluorescent Carbon Nanodots.

Patent application number: 202011021910

Published Articles (Thesis)

1. **Ashish Tiwari**, Navneet C. Verma, Anup Singh, Chayan K. Nandi, and Jaspreet K. Randhawa. "Carbon Coated Core–Shell Multifunctional Fluorescent SPIONs." *Nanoscale* 10, (2018): 10389-10394.

2. **Ashish Tiwari**, Ashutosh Singh, Ayan Debnath, Ankur Kaul, Neha Garg, Rashi Mathur, Anup Singh, and Jaspreet K. Randhawa. "Multifunctional Magneto-Fluorescent Nanocarriers for Dual Mode Imaging and Targeted Drug Delivery." *ACS Applied Nano Materials* 2, (2019): 3060-3072.

3. **Ashish Tiwari**, Navneet C. Verma, Jaspreet K. Randhawa, and Chayan K. Nandi. "Real-Time Observation of Magnetic Field-Induced Fluorescence Engineering in SPIONs." *The Journal of Physical Chemistry C* 123, (2019): 27759-27764.

4. **Ashish Tiwari**, Navneet Chandra Verma, Sibel Turkkan, Ayan Debnath, Anup Singh, Gerald Draeger, Chayan Kanti Nandi, and Jaspreet Kaur Randhawa. "Graphitic Carbon Coated Magnetite Nanoparticles for Dual-Mode Imaging and Hyperthermia." *ACS Applied Nano Materials* 3, (2020): 896-904.

5. **Ashish Tiwari**, Raj Kumar, Orit Shefi and Jaspreet Kaur Randhawa, "Fluorescently Mantled Carbon Coated Core-Shell SPIONs for Neuroengineering Applications." *ACS Applied Bio Materials* 2020.

6. **Ashish Tiwari**, Prachi Bhatia and Jaspreet Kaur Randhawa, "Systematic Spectroscopic Investigation of Structural Changes and Corona Formation of Bovine Serum Albumin over Magneto-Fluorescent Nanoparticles." *RSC-Dalton Transactions* 2020.

Abbreviations

AA	Ascorbic acid
AIEE	Aggregation induced emission enhancement
AMF	Alternating magnetic field
AMF	Applied magnetic field
AES	Auger electron spectroscopy
AFM	Atomic force microscopy
ATR	Attenuated total reflection
BALB/c	An albino, immunodeficient inbred strain of the house mouse
BET	Brunauer–Emmett–Teller
BJH	Barrett-Joyner-Halenda
BSA	Bovine Serum Albumin
C	Celsius
CCD	Charge coupled device
CHI	Chitosan
CLEM	Correlative light and electron microscopy
CLSM	Confocal laser scanning microscopy
CNs	Carbon nanostructures
CPCSEA	Committee for Purpose of Control and Supervision of Experiments on Animals
CT	Computed tomography
CY	Cyanine dyes
D	Drag coefficient
DI	Deionized
DLC	Drug loading capacity
DLS	Dynamic light scattering
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DR	Drug release

EC	Echo time
EDC	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
EDTA	Ethylene diamine tetraacetic acid
EM	Electron microscope
EMR	Electromagnetic radiation
EMCCD	Electron multiplying charge-coupled device
EPR	Enhanced permeation and retention
eV	Electron volt
FA	Folic acid
FBS	Fetal bovine serum
FC	Field cooling
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscopy
FFT	Fast Fourier transform
FCNDs	Fluorescent carbon nanodots
FTIR	Fourier transform infrared spectroscopy
FU	Fluorouracil
FOV	Field of view
FPS	Frames per second
FWHM	Full width half maximum
HAADF	High-angle annular dark-field imaging
HRMS	High resolution mass spectrometry
HRTEM	High resolution transmission electron microscopy
Hz	Hertz
ICDD	International center for diffraction data
ID/gm	Injected dose per gram
ITLC	Instant thin layer chromatography
ILP	Intrinsic loss power

IV	Intravenous injection
JCPDS	Joint committee on powder diffraction standards
K	Kelvin
KA/m	Kiloampere per metre
kDa	Kilodalton
kV	Kilovolt
kW	Kilowatt
kHz	Kilohertz
LFG	linear filed gradients
mA	Milliampere
mM	Millimolar
MF	Magnetic field
MFE	Magnetic field effect
MFCSNPs	Multifunctional carbon coated core shell SPIONs
Mg/ml	Milligram per milliliter
MgCl ₂	Magnesium chloride
MH	Magnetic hyperthermia
MHz	Megahertz
ml	Millilitre
mm	Millimetre
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MRI	Magnetic resonance imaging\
Ms	Millisecond
mΩ	Milliohm
MV	Methyl viologen
NA	Numerical aperture
NaCl	Sodium chloride
NaHCO ₃	Sodium hydrogen carbonate

NaOH	Sodium hydroxide
NBCF	National Breast Cancer Foundation
NCCS	National Centre for Cell Science
NHS	N-hydroxysuccinimide
nm	Nanometer
nM	Nanomolar
NMR	Nuclear magnetic resonance
PALM	Photoactivated localization microscopy
PBS	Phosphate buffered saline
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PET	Positron emission tomography
PH	Potential of Hydrogen
PMT	Photomultiplier tube
PSF	Point spread function
PTT	Photothermal therapy
QDs	Quantum dots
QY	Quantum yield
R ₂	Relaxivity
RB	Rhodamine dye
RBC	Red blood cells
RF	Radiofrequency pulsed
ROI	Region of interest
SAED	Selected area electron diffraction
SAR	Specific absorption rate
SDS	Sodium Dodecyl Sulfate
SEM	Scanning electron microscopy
SLP	Specific loss power

SMLM	Single molecule localization based methods
SPFI	Single particle fluorescence imaging
SPIONs	Superparamagnetic iron oxide nanoparticles
SPT	Single particle tracking
SQUID	Superconducting quantum interference device
STED	Stimulated emission depletion
STEM	Scanning transmission electron microscopy
STORM	Stochastic optical reconstruction microscopy
T	Tesla
Tc	Technetium-99m
TDD	Targeted drug delivery
TE	Echo time
TR	Repetition time
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TIRF	Total internal reflection fluorescence
TX	Trolox
U	Dipole-dipole interaction energy
USPSTF	United States Preventive Services Task Force
UV-Vis	Ultraviolet visible
VSM	Vibrating sample magnetometer
W/g	Watt/gram
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
μL	Microliter
μm	Micrometre
μCi	Micro curie
ZFC	Zero field cooling

Table of Content

1	Introduction.....	1
1.1	Opening remarks	3
1.2	Objective and overview of the thesis	4
1.3	Introduction.....	5
1.3.1	Cancer theranostics: Global statistics, market size and share and modern trends and analysis.....	6
1.3.2	Magnetic nanoparticles: Global statistics, market size and share modern trends and analysis	9
1.4	Overview of nanostructures	11
1.4.1	Magnetic nanoparticles (Superparamagnetic iron oxide nanoparticles (SPIONs))	11
1.4.2	Fluorescent nanoparticles (Carbon nanostructure).....	13
1.4.3	Magneto-fluorescent nanoparticles	15
1.4.4	Engineering of nanostructures: A hybrid approach to multifunctional nanoparticles	17
1.5	Overview of imaging modalities in cancer theranostics.....	19
1.5.1	Magnetic resonance (MR) imaging	19
1.5.2	Fluorescence imaging	22
1.5.3	Single particle fluorescence imaging (TIRF imaging).....	24
1.5.4	Single particle tracking	27
1.5.5	Combined multimodal magnetic resonance and fluorescence imaging modalities	30
1.6	Overview of therapeutic modalities in cancer theranostics	33
1.6.1	Magnetic hyperthermia	33
1.6.2	Combined multimodal imaging and hyperthermia.....	38
1.6.3	Drug delivery.....	39
1.6.4	Targeted drug delivery (Active and passive drug delivery).....	41
1.6.5	Combined multimodal imaging and targeted drug delivery.....	43
1.6.6	Cancer theranostics.....	44
1.7	Spectroscopic interaction studies with human serum protein	45
1.8	Neuroengineering and neuronal network outgrowth studies	47
1.9	Magnetic field induced fluorescence engineering studies.....	49
1.10	Overview of the chapters	51
1.11	References.....	54
2	Materials and Methods.....	68
2.1	Materials	70
2.2	Material synthesis methods	71
2.2.1	Synthesis of multifunctional magneto-fluorescent core shell nanostructures	71
2.2.2	Synthesis of morphology tuned three multifunctional magneto-fluorescent core shell nanostructures	71
2.2.3	Synthesis of folic acid and chitosan (FA-CHI) conjugates	72

2.2.4	¹ H NMR spectroscopy studies of folic acid and chitosan (FA-CHI) conjugates	72
2.2.5	Synthesis of multifunctional magneto-fluorescent nanocarriers (MFCSNPs-FA-CHI-5FU nanocarriers)	73
2.3	Material Characterization Techniques.....	73
2.3.1	Transmission electron microscopy (TEM) imaging studies.....	73
2.3.2	Field emission scanning electron microscopy (FESEM) imaging studies	74
2.3.3	Atomic force microscopy (AFM) imaging studies	75
2.3.4	X-Ray diffraction (XRD) spectroscopic studies	76
2.3.5	X-ray photoelectron (XPS) spectroscopic studies.....	76
2.3.6	Confocal laser Raman spectroscopic studies	77
2.3.7	UV-visible (UV-Vis) spectroscopic studies.....	78
2.3.8	Fourier transform infrared (FTIR) spectroscopic studies.....	79
2.3.9	Thermogravimetric analysis (TGA) studies.....	79
2.3.10	Nuclear magnetic resonance (NMR) spectroscopic studies	80
2.3.11	Brunauer–Emmett–Teller (BET) analysis studies.....	81
2.3.12	Dynamic light scattering (DLS) measurements studies	81
2.3.13	Fluorescence spectroscopy and fluorescence lifetime measurements studies	82
2.3.14	Quantum yield (QY) calculation.....	83
2.4	Magnetic properties and Magnetic resonance (MR) imaging studies	84
2.4.1	Magnetic property analysis studies	84
2.4.2	Phantom preparation for magnetic resonance (MR) imaging studies	85
2.4.3	MR imaging and relaxivity studies	85
2.4.4	Relaxivity mapping.....	85
2.5	Magnetic hyperthermia studies	86
2.5.1	Alternating magnetic field (AMF) hyperthermia studies	86
2.5.2	Specific loss power (SLP) measurement and calculation studies.....	87
2.6	Biological studies	87
2.6.1	Drug loading estimation and calculation studies.....	87
2.6.2	In-vitro drug release studies.....	87
2.6.3	Human cancer cell culture studies	88
2.6.4	In-vitro cytotoxicity studies	88
2.6.5	In-vitro targeted cellular internalization studies.....	89
2.6.6	In-vitro targeted MR imaging studies	90
2.6.7	In-vitro hemolysis studies.....	90
2.6.8	In-vitro serum stability studies	91
2.6.9	Radiolabeling studies.....	92
2.6.10	Animal evaluation studies.....	92
2.6.11	Animal handling and in-vivo biodistribution studies	93
2.7	Spectroscopic investigation studies with serum protein (BSA)	93

2.7.1	Fluorescence quenching studies (Steady state fluorescence spectroscopy and lifetime and anisotropy measurements)	93
2.7.2	Functional group, conformational analysis, thermal stability and dynamic size distribution analysis studies	94
2.7.3	Circular dichroic (CD) measurement and Raman analysis studies.....	94
2.8	Neuroengineering experiments studies (Cell culture, optical imaging and SEM imaging).....	95
2.8.1	Cell culture studies	95
2.8.2	Cell viability assays	95
2.8.3	PC12 cell differentiation studies.....	96
2.8.4	Scanning electron microscopy (SEM) imaging of neural network	96
2.8.5	Immunofluorescence staining and confocal imaging studies	97
2.9	Single particle fluorescence microscopy studies	98
2.9.1	Total internal reflection fluorescence microscopy (TIRF).....	98
2.9.2	Single particle fluorescence (SPF) imaging studies	98
2.9.3	Single particle tracking (SPT) analysis studies	99
2.9.4	Single particle fluorescence imaging (SPFI) under the applied magnetic field	100
2.9.5	Preparation of agarose gel embedded SPIONs and self-assembled SPIONs	100
2.9.6	FESEM and single particle imaging of agarose gel embedded SPIONs and self-assembled SPIONs.....	100
2.9.7	Calculation of the Magnetic field effect (MFE).....	100
2.9.8	Calculation of the dipole-dipole interaction energy (U)	101
2.9.9	Calculation of the drag coefficient (D)	101
2.10	References	103
3	Magneto-fluorescent carbon coated core shell SPIONs nanoarchitectures for multimodal imaging....	105
3.1	Abstract	107
3.2	Introduction.....	107
3.3	Results and Discussion.....	110
3.3.1	Formation of carbon coated core shell multifunctional fluorescent SPIONs and fluorescent carbon nanodots in facile single step synthesis	110
3.3.2	Morphological and structural analysis of fluorescent carbon nanodots	112
3.3.3	Morphological analysis studies of MFCSNPs	116
3.3.4	Structural integrity and functional group analysis studies	118
3.3.5	Optical properties studies.....	121
3.3.6	Confocal imaging and multicolour behaviour studies.....	122
3.3.7	Magnetic properties analysis studies.....	123
3.3.8	Magnetic resonance imaging studies	125
3.3.9	Single particle fluorescence imaging studies	126
3.4	Conclusion	129
3.5	References	130

4	Structurally tuned magneto-flourescent carbon coated core shell SPIONs nanoarchitectures for multimodal imaging and magnetic hyperthermia.....	134
4.1	Abstract	136
4.2	Introduction.....	136
4.3	Results and Discussion.....	138
4.3.1	Fabrication of shell thickness controlled graphitic carbon coated core shell SPIONs	138
4.3.2	Morphological and particle size analysis studies	141
4.3.3	Crystalline structure, lattice phase and functional group analysis studies.....	144
4.3.4	Fluorescence and lifetime spectroscopic analysis studies	148
4.3.5	Single particle fluorescence imaging analysis studies	149
4.3.6	Magnetic properties analysis studies.....	151
4.3.7	Magnetic resonance imaging analysis studies.....	153
4.3.8	Magnetic hyperthermia analysis studies	155
4.4	Conclusion	158
4.5	References	159
5	Multifunctional magneto-flourescent carbon coated core shell SPIONs for targeted drug delivery in cancer theranostics	164
5.1	Abstract	166
5.2	Introduction.....	166
5.3	Results and Discussion.....	169
5.3.1	Morphological and structural analysis	169
5.3.2	Crystalline structure and phase analysis studies	173
5.3.3	Formation of folic acid and chitosan conjugates (¹ HNMR and FTIR spectra studies).....	173
5.3.4	Functional group analysis studies	176
5.3.5	Colloidal stability and hydrodynamic size distribution analysis studies	177
5.3.6	Specific surface area and pore size distribution analysis studies	178
5.3.7	Optical property analysis studies	179
5.3.8	Thermal stability analysis studies	180
5.3.9	Fluorescence properties analysis studies.....	181
5.3.10	Magnetic properties analysis studies.....	181
5.3.11	Drug loading estimation and release studies	182
5.3.12	Magnetic resonance (MR) imaging studies.....	184
5.3.13	In-vitro targeted MR imaging studies	185
5.3.14	In-vitro cytotoxicity studies	186
5.3.15	Cellular uptake and internalization studies	188
5.3.16	In-vitro targeted cellular internalization studies on cancer cells	189
5.3.17	In-vitro hemolysis studies.....	191
5.3.18	In-vitro serum stability analysis studies.....	192

5.3.19	In-vivo biodistribution and in-vivo animal evaluation studies.....	193
5.4	Conclusion	195
5.5	References	196
6	Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for protein sensing and neuroengineering applications.....	201
6.1	Abstract	203
6.2	Introduction.....	203
6.3	Results and Discussion.....	205
6.3.1	Synthesis of magneto-fluorescent nanoparticles (MFNPs).....	205
6.3.2	Synthesis of magneto-fluorescent nanoparticles (MFNPs).....	205
6.3.2.1	Effect on the emission spectra of BSA with addition of the MFNPs	205
6.3.2.2	Association constant (K _a) and number of binding sites (n)	208
6.3.2.3	Thermodynamic parameters calculation (Determination of the binding forces)	208
6.3.4	Conformational change studies.....	210
6.3.4.1	UV-Vis absorption spectroscopic studies	210
6.3.4.2	Raman spectroscopic studies	210
6.3.4.3	Time correlated single photon counting fluorescence and fluorescence anisotropy studies	211
6.3.4.4	Circular dichroism (CD) spectroscopic studies.....	213
6.3.4.5	Mechanism of fluorescence quenching of BSA in the presence of MFNPs.....	216
6.3.5	Spectroscopic investigations of protein corona formation over MFNPs.....	218
6.3.5.1	Transmission electron microscopy (TEM) studies.....	218
6.3.5.2	Effect of BSA on the surface charge and hydrodynamic diameter of MFNPs.....	218
6.4	Magneto-fluorescent nanoparticles for neuroengineering applications.....	220
6.4.1	Interaction of MFNPs with neuronal cells (PC12 cells).....	220
6.4.2	Cell viability studies (In vitro cytotoxicity studies).....	221
6.4.3	Neuronal cell differentiation and optical imaging studies.....	223
6.4.4	SEM imaging of neuronal network outgrowth.....	224
6.4.5	Cellular internalization and confocal imaging studies	226
6.5	Conclusion	228
6.6	References	229
7	Real time observation of magnetic field induced self-assembly and fluorescence engineering in SPIONs using single particle fluorescence imaging and tracking	233
7.1	Abstract	235
7.2	Introduction.....	235
7.3	Results and Discussion.....	237
7.3.1	Overview of the properties of the fluorescent SPIONs.....	237
7.3.2	Magnetic field induced fluorescence enhancement in bulk measurements	238
7.3.3	Single particle imaging studies of fluorescence enhancement under applied magnetic field.....	239

7.3.4	Single particle tracking of dynamics and self-assembly of SPIONs under applied magnetic field.....	242
7.3.5	Mechanism of self-assembly and associated fluorescence enhancement under applied magnetic field.....	243
7.3.6	Fortitude of various factors governing the self-assembly and associated fluorescence enhancement under applied magnetic field.....	244
7.3.7	Estimation and calculation of the dipole-dipole interaction energy and drag coefficient.....	246
7.3.8	Proof of concept of magnetic field induced AIEE based fluorescence enhancement in SPIONs	249
7.4	Conclusion	251
7.5	References	251
8	Conclusion and Future work	255
8.1	Summary and overall conclusion of the dissertation.....	257
8.2	Future prospects of the work.....	259
9	Appendix.....	262