

List of Figures

List of Figures

Chapter 1	Page No.
Figure 1.1: Major barriers in nanoparticle delivery to malignant glioma.	5
Figure 1.2: Efflux transporters expressed on BBB.	6
Figure 1.3: Transportation mechanisms of multifunctional nanoparticles into the brain tumor.	9
Figure 1.4: Representation of brain cancer targeted nanoparticles system.	11
Figure 1.5: Schematic representation of theranostic nanoparticles.	12
Figure 1.6: Nose to brain transfer of drug via. Olfactory pathway	14
Chapter 2	Page No.
Figure 2.1: Multifunctional Metal Nanoparticles	34
Figure 2.2: Chemical structure of Lenalidomide	37
Figure 2.3: Mechanism of action of Lenalidomide	39
Figure 2.4: Multifunctional nanoparticles targeted to cancer cell membranes using a ligand to tumor cell specific surface receptor.	43
Figure 2.5: Biomedical application of FePt nanoparticles	50
Chapter 3	Page No.
Figure 3.1: Mechanism of Azo-dye formation	94
Figure 3.2: Standard plot of LND in PBS 7.4 by UV visible spectroscopy	95
Figure 3.3: Standard plot of LND in CPB 5.5 by UV visible spectroscopy	96
Figure 3.4: Standard plot of LND in Methanol by UV visible spectroscopy	99
Figure 3.5: Standard plot of Iron in distilled water by UV visible spectroscopy	100
Figure 3.6: Scheme for the reaction pathway between LND and Fluorescamine.	102
Figure 3.7: Standard plot of LND in plasma by spectrofluorimetry	103
Figure 3.8: Overlay spectra of LND in plasma by spectrofluorimetry	103
Figure 3.9: UV spectra of (1) LND, (2) Mixture of LND and HAand (3) Mixture of LND and CMHA in PBS 7.4 for interference study.	104

List of Figures

Chapter 6	Page No.
Figure 6.1: Ishikawa diagram for selection of factors	147
Figure 6.2: Preliminary investigation for effect of reflux temperature and time on particle size	149
Figure 6.3: Effect of (A) reflux time and temperature on platinum atomic composition (At% Pt) in FePt nanoparticles; (B) reflux time and temperature on platinum atomic composition (At% Fe) in FePt nanoparticles	149
Figure 6.4: Effect of (A) 1,2-Hexadecanediol and reflux time on particle size; (B); 1,2-Hexadecanediol and reflux temperature on particle size; (C) 1,2-Hexadecanediol and reflux time on atomic composition of Pt (At% Pt); (D) 1,2-Hexadecanediol and reflux time on atomic composition of Fe (At% Fe); (E) 1,2-Hexadecanediol and reflux temperature on atomic composition of Pt (At% Pt); (F) 1,2-Hexadecanediol and reflux temperature atomic composition of Fe (At% Fe) in FePt nanoparticles	150
Figure 6.5: Half normal plot obtained for Y1 (A), Y2 (B) and Y3 (C) for synthesized FePt nanoparticles	154
Figure 6.6: Statistical analysis of Y1 (A), Y2 (B) and Y3 (C) for synthesized FePt nanoparticles	155
Figure 6.7: Parameter estimates for Y1 (A), Y2 (B) and Y3 (C) for synthesized FePt nanoparticles	156
Figure 6.8: Contour plots showing (A) effect of X2 and X1 on Y1; (B) effect of X3 and X1 on Y1; (C) effect of X3 and X2 on Y1 for synthesized FePt nanoparticles	157
Figure 6.9: Contour plots showing effect of (A) X3 and X1 on atomic percent of Fe (Iron); (B) X3 and X2 on atomic percent of Fe; (C) X2 and X1 on atomic percent of Fe; (D) X3 and X1 on atomic percent of Pt (Platinum); (E) X3 and X1 on atomic percent of Pt; (F) X3 and X1 on atomic percent of Pt in synthesized FePt nanoparticles	158
Figure 6.10: Desirability plot obtained for synthesis of FePt nanoparticles	159
Figure 6.11: Effect of factors on % Fe in FePt nanoparticles	160
Figure 6.12: Effect of factors on particle size of FePt nanoparticles	160
Figure 6.13: Mechanism of phase transfer of hydrophobic FePt nanoparticles to	163

List of Figures

hydrophilic phase.

- Figure 6.14: FT-IR graph for (A) oleic acid coated and (B) uncoated FePt nanoparticles 165
- Figure 6.15: FTIR spectra of unmodified FePt nanoparticles (A), amine modified FePt nanoparticles (B), oleic acid coated FePt nanoparticles (C), and B-FePt nanoparticles (D). 168
- Figure 6.16: Characterization of FePt nanoparticles using (A) XRD; (B) XPS analysis of Fe in FePt nanoparticles; (C) XPS analysis of Pt in FePt nanoparticles 169
- Figure 6.17: EDAX of FePt-NH₂ nanoparticles 170
- Figure 6.18: XRD peaks of (A) FePt and (B) Fe₂O₃@FePt nanoparticles 175
- Figure 6.19: EDAX of Fe₂O₃@FePt nanoparticles 176
- Figure 6.20: TEM of (A) FePt and (B) Fe₂O₃@FePt nanoparticles; STEM image of (C) Fe₂O₃@FePt nanoparticles showing presence of Fe₂O₃ over FePt nanoparticles; (D) Pt in Fe₂O₃@FePt nanoparticles (blue dots); (E) Fe (red dots) in Fe₂O₃@FePt nanoparticles. 176
- Figure 6.20: XPS of Fe₂O₃@FePt nanoparticles; (A) Pt 4f of FePt core; (B) Fe 2p of FePt core; (C) Fe 2p of Fe₂O₃ shell. 177
- Figure 6.22: AFM images of (A) FePt (6.43nm); (B) Fe₂O₃@FePt (9.4nm) nanoparticles 178
- Figure 6.23: Magnetic hysteresis of curve of (A) FePt and (B) Fe₂O₃@FePt nanoparticles 178
- Figure 6.24: Raman shift for (A) FePt nanoparticles; (B) Fe₂O₃@FePt nanoparticles. 179
- Figure 6.25: In vitro generation of heat in FePt nanoparticles suspension in response to applied magnetic field (A), NIR laser treatment (808nm) (B), NIR laser treatment (1064nm) (C), NIR laser treatment+335.2Oe magnetic field (D) and in vitro heat generation in presence of applied magnetic field (335.2 Oe), NIR laser treatment (808 and 1064 nm) and combined magnetic field (335.2 Oe) and NIR laser treatment (808 nm and 1064nm) (E). 181
- Figure 6.26: In vitro generation of heat in Fe₂O₃@FePt nanoparticles suspension in response to applied magnetic field (A), NIR laser treatment (808nm) (B), NIR laser 183

List of Figures

treatment (1064nm) (C), NIR laser treatment+335.2Oe magnetic field (D) and in vitro heat generation in presence of applied magnetic field (335.2 Oe), NIR laser treatment (808 and 1064 nm) and combined magnetic field (335.2 Oe) and NIR laser treatment (808 nm and 1064nm) (E).

Figure 6.27: Endosomal localization of FePt nanoparticles in U87MG cells	184
Figure 6.28: FT-IR spectrum of hyaluronic acid	186
Figure 6.29: FT-IR spectrum of CMHA	187
Figure 6.30: NMR spectra of (A) HA; (B) CMHA	188
Figure 6.31: FTIR spectra of CMHA-LND conjugate	190
Figure 6.32: NMR spectrum of CMHA-drug conjugate	191
Figure 6.33:(A) DSC curve of (A) LND; (B) Hyaluronic acid; (C) Fe ₂ O ₃ @FePt-HA@LND nanoconjugates; (B) DSC curve of CMHA-LND conjugates	192
Figure 6.34: XRD plot of (A) LND, (B) CMHA, (C) CMHA-LND Conjugates, and (D) Fe ₂ O ₃ @FePt-CMHA-LND conjugates.	192
Figure 6.35: ESR curves for (A) Fe ₂ O ₃ @FePt Nanoparticles and (B) Fe ₂ O ₃ @FePt-CMHA-LND nanoparticles	193
Figure 6.36: Graph showing (A) drug release and (B) release of Fe from Fe ₂ O ₃ @FePt nanoparticles.	195

Chapter 7

Page No.

Figure 7.1: Outline for carrying out In vitro BBB passage study with and without AMF.	209
Figure 7.2: <i>In vitro</i> cell viability study in U87MG cells using FePt, LND and SPANs after 24 h (A) and 48 h (B).	217
Figure 7.3: Photothermal killing using SPANs (placebo) (C), chemo-photothermal killing using SPANs (D), chemo-magnetophotothermal killing using SPANs (E). Comparative cell viability has been shown for multimodal killing of U87MG cells (F).	218
Figure 7.4: <i>In vitro</i> cell viability study in U87MG cells using surface modified Fe ₂ O ₃ @FePt nanoparticles after 24 h.	220
Figure 7.5: <i>In vitro</i> BBB passage studies for pure LND, Fe ₂ O ₃ @FePt and SPANs after	221

List of Figures

2h, 24h and 48h without AMF.

Figure 7.6: *In vitro* BBB passage studies for pure LND, Fe₂O₃@FePt and SPANs after 2h, 24h and 48h with AMF. 221

Figure 7.7: *In vitro* BBB passage studies for pure LND, Fe₂O₃@FePt and SPANs after 24h with AMF at concentration of 2mg/mL, 5mg/mL and 10mg/mL 222

Figure 7.8: Quantitative estimation of surface ROS generation by LND, Fe₂O₃@FePt and SPANs in U87MG cells. 223

Figure 7.9: Quantitative estimation of ROS generation in U87MG cells at different concentration of Fe₂O₃@FePt and SPANs at 25µg/mL with AMF exposure for 5 and 10 minutes respectively. 224

Figure 7.10: Colony forming assay of FePt nanoparticles, Fe₂O₃@FePt nanoparticles and SPANs qualitative (A); quantitative (B). 225

Figure 7.11: Cellular internalization of SPANs as visualized using Prussian blue staining after 12 (A), 24 (B) and 48 h (C) along with nuclear localization of SPANs after 48h (D). The quantitative estimation of SPANs in U87MG cells was done at two different dosing concentrations of 0.8mg/mL and 1.2mg/mL after 12, 24 and 48 h respectively (E), presence of SPANs inside cells was also analyzed till 72 h (F). 226

Figure 7.12: Cellular internalization of Fe₂O₃@FePt as visualized using Prussian blue staining after 12 (A), 24 (B) and 48 h (C) along with control 48h (D) in U87MG cells done at two different dosing concentrations of 0.8mg/mL and 1.2mg/mL after 12, 24 and 48 h respectively. 227

Figure 7.13: Confocal microscopic images of U87MG cells after being treated with FITC-M-SPANs (25µg/mL). (Mitochondria is visualized in red). 229

Figure 7.14: Confocal microscopic images of U87MG cells after being treated with FITC- SPANs (25µg/mL). (Mitochondria are visualized in red). 230

Figure 7.15: Assessment of mitochondrial targeting ability of SPANs and M-SPANs in isolated mitochondria 231

Figure 7.16: (A) Mitochondrial membrane potential detection after treating U87MG cells with FCCP (positive control), SPANs and M-SPANs (50µg/mL) for 24h. (B)Flow cytometry analysis after treating U87MG cells with FCCP (positive control), 232

List of Figures

SPANs and M-SPANs (50 μ g/mL) for 24h. FLI-TMRE means fluorescence intensity of TMRE.

Figure 7.17: Cellular ATP level of U87MG cells after being treated with SPANs and M-SPANs for 24h at concentration of 25 μ g/mL Oligomycin acts as positive control. 233

Figure 7.18: Activity of mitochondrial respiratory chain complexes in U87MG cells in presence of SPANs and M-SPANs. 233

Chapter 8

Page No.

Figure 8.1: Concentration of drug in plasma after administration by intranasal route 245

Figure 8.2: Concentration of drug in plasma after administration by intravenous route 246

Figure 8.3: A comparative bar graph representation of drug concentration v/s time profile in blood and brain after intranasal and intravenous administration of M-SPANs 249

Figure 8.4: Percent transfer of FITC conjugated M-SPANs across nasal mucosa (1), in vivo nasal penetration of M-SPANs (2A-D) after 15, 30, 60 and 120 min respectively. Accumulation of M-SPANs in brain after nasal administration was assessed qualitatively after 15 min (8.4-3(A)), 30 min (8.4-3(B)), 60 min (8.4-3(C)) and 120 min (8.4-3(D)) using fluorescence microscopy, while quantitative estimation of SPANs in brain was done using ICP-AES (8.4-4). 250

Figure 8.5: Histopathological images of isopropyl alcohol as positive control (a), PBS (pH 6.4) as negative control (b), LND drug solution (c) and M-SPANs (d) 252

Figure 8.6: % administered activity after 3h, 1 day and 3 day in rats 253

Figure 8.7: Assessment of interaction between M-SPANs and bio-molecules (plasma, mucin and ECM) 254

Figure 8.8: Activity of mitochondrial respiratory chain complexes in brain homogenate in presence of SPANs and M-SPANs 255

Figure 8.9: Assessment of interaction between M-SPANs and bio-molecules (plasma, mucin and ECM) 257