

List of Figures

Figure 1.1 Pathogenesis of Atherosclerosis.....	2
Figure 2.1 Plaque formations in atherosclerosis.....	23
Figure 2.2 Stages of development of plaque.....	26
Figure 2.3 Schematic structures of lipoplex, lipopolyplex and lipid nanoparticle based delivery systems.....	48
Figure 2.4 Schematic structures of micelles, solid lipid nanoparticle and nanoemulsion based delivery systems.....	49
Figure 3.1 UV absorption profile of isolated pDNA.....	92
Figure 3.2 Correlation of actual concentration of pDNA vs observed concentration.....	93
Figure 3.3 pDNA standard curve using spectrofluorimetry.....	95
Figure 3.4 Determination of quantifiable range of pDNA.....	99
Figure 3.5 Band densities at 200 ng Lane 1 to Lane 6: 200 ng pDNA.....	99
Figure 3.6 Calibration plot of DNA gel retardation.....	101
Figure 3.7 Reaction of primary amine containing compound with TNBS.....	102
Figure 3.8 Overlay spectra of TNBS assay for stearyl amine.....	104
Figure 3.9 Calibration curve of TNBS assay of stearyl amine.....	104
Figure 3.10 Overlay spectra of TNBS assay of DSPE A) Left pane-1 hr reaction time B) Right panel-6 hr reaction time.....	106
Figure 3.11 Calibration curve of TNBS assay of DSPE at 340 nm.....	106
Figure 3.12 Overlay spectra of Sakaguchi assay of Boc-arginine (X-axis: Absorbance, Y-axis: wavelength (nm)).....	108
Figure 3.13 Calibration curve of Sakaguchi assay of Boc-arginine.....	109

Figure 4.1 Schematic diagram representing the streaked agar plates with different E. Coli.	122
Figure 4.2 Flow chart representing the alkaline lysis and isolation method of Qiagen kit...	125
Figure 4.3 Chemical structures of the Qiagen anion exchange resin and DNA showing their respective binding sites.....	125
Figure 4.4 Restriction Digestion of APOE3 pDNA.....	131
Figure 4.5 Restriction digestion of eGFP pcDNA by HindIII enzyme.....	132
Figure 5.1 Synthesis of histidine conjugated DSPE.....	135
Figure 5.2 Synthesis of Boc-carnosine.....	136
Figure 5.3 Structure of DSPE and DSPE based lipids (Value at amine groups indicates the calculated pKa of that group according to Chemaxon (Chemicalize.org).....	140
Figure 5.4 UV spectra Carnosine and carnosine isolated after Boc protection	142
Figure 5.5 NMR spectra of Carnosine and Boc-Carnosine.....	142
Figure 5.6 IR spectra of Carnosine and Boc-Carnosine.....	143
Figure 5.7 UV spectra of DSPE, Boc-Car and Boc-Car-DSPE (x-axis: wavelength, y-axis: absorbance).....	143
Figure 5.8 UV spectra of DSPE, Boc-His and Boc-His-DSPE (x-axis: wavelength, y-axis: absorbance).....	144
Figure 5.9 pH titration curve of Boc-Histidine, Boc-His-DSPE and His-DSPE	145
Figure 5.10 pH titration curve of Carnosine, Boc-Carnosine, Boc-Car-DSPE and Car-DSPE	146
Figure 5.11 pH titration curve of DSPE and Arg-DSPE.....	146

Figure 6.1 Lyophilization Cycle	152
Figure 6.2 Temperature Change during Lyophilization	152
Figure 6.3 Intensity-weighted particle size distribution of Batch B1	163
Figure 6.4 Intensity-weighted particle size distribution of Batch B2	163
Figure 6.5 Intensity-weighted particle size distribution of Batch B3	163
Figure 6.6 Intensity-weighted particle size distribution of Batch B4	164
Figure 6.7 Intensity-weighted particle size distribution of Batch B5	164
Figure 6.8 Intensity-weighted particle size distribution of Batch B6	164
Figure 6.9 Intensity-weighted particle size distribution of Batch B7	165
Figure 6.10 Intensity-weighted particle size distribution of Batch B8	165
Figure 6.11 Intensity-weighted particle size distribution of Batch B9	165
Figure 6.12 Intensity-weighted particle size distribution of Batch B10	166
Figure 6.13 Intensity-weighted particle size distribution of Batch B11	166
Figure 6.14 Intensity-weighted particle size distribution of Batch B12	166
Figure 6.15 Intensity-weighted particle size distribution of Batch B13	167
Figure 6.16 Intensity-weighted particle size distribution of Batch C1	171
Figure 6.17 Intensity-weighted particle size distribution of Batch C2	171
Figure 6.18 Intensity-weighted particle size distribution of Batch C3	171
Figure 6.19 Intensity-weighted particle size distribution of Batch C4	171
Figure 6.20 Intensity-weighted particle size distribution of Batch C5	172
Figure 6.21 Intensity-weighted particle size distribution of Batch C7	172
Figure 6.22 Intensity-weighted particle size distribution of Batch C8	172
Figure 6.23 Intensity-weighted particle size distribution of Batch C9	173
Figure 6.24 Intensity-weighted particle size distribution of Batch C10	173
Figure 6.25 Intensity-weighted particle size distribution of Batch C11	173
Figure 6.26 Intensity-weighted particle size distribution of Batch C13	174
Figure 6.27 Intensity-weighted particle size distribution of Batch C14	174
Figure 6.28 Intensity-weighted particle size distribution of Batch C15	174
Figure 6.29 Intensity-weighted particle size distribution of Batch C16	175
Figure 6.30 Intensity-weighted particle size distribution of Batch C17	175
Figure 6.31 TEM image of liposomes of Batch C10	175
Figure 6.32 TEM image of liposomes of Batch C13	176
Figure 6.33 TEM image of liposomes of Batch C17	176
Figure 6.34 Model diagnostic plots	183
Figure 6.35 Box-Cox plot of power transformation	184
Figure 6.36 Residual vs. factor plots for determining the time-independence of variance	185
Figure 6.37 Piepel's plot	186
Figure 6.38 Two-component mixture plots ; A: effect of HSPC and EPC, B: effect of HSPC and Chol, C: effect of EPC and Chol	187
Figure 6.39 Contour plot of effects of different components on particle size	189

Figure 6.40 Response surface plot of effects of different components on particle size ...	190
Figure 6.41 Model diagnostic plots	193
Figure 6.42 Box-Cox plot of power transformation	194
Figure 6.43 Residual vs. factor plots for determining the time-independence of variance	196
Figure 6.44 Piepel's plot.....	196
Figure 6.45 Two-component mixture plots; A: effect of HSPC and EPC, B: effect of HSPC and Chol, C: effect of EPC and Chol.....	197
Figure 6.46 Contour plot of effects of different components on PDI.....	199
Figure 6.47 Response surface plot of effects of different components on PDI.....	199
Figure 6.48 Surface plots showing optimum particle size and PDI at best trade-off for the constraints	201
Figure 6.49 Desirability Plot for Selection of Optimized Batch.....	202
Figure 6.50 Design space with optimum response parameters.....	203
Figure 6.51 Intensity-weighted particle size distribution of non-PEGylated (Red) and PEGylated (Green) liposomes.....	205
Figure 6.52 Intensity-weighted particle size distribution of HDS liposomes (Red) and BHDS liposomes (Green)	206
Figure 6.53 Intensity-weighted particle size distribution of BCDS liposomes (Red) and CDS liposomes (Green)	206
Figure 6.54 Intensity-weighted particle size distribution of ADS liposomes (Red) and BADS liposomes (Green)	206
Figure 6.55 Complexation efficiency of non-PEGylated lipoplexes – 30 min incubation	208
Figure 6.56 Complexation efficiency of non-PEGylated lipoplexes – 45 min incubation	209
Figure 6.57 Complexation efficiency of PEGylated lipoplexes 30 min incubation at 25°C	210
Figure 6.58 Complexation efficiency of PEGylated lipoplexes 45 min incubation at 25°C	210
Figure 6.59 Complexation efficiency of PEGylated lipoplexes 60 min incubation at 25°C	210
Figure 6.60 Complexation efficiency of PEGylated lipoplexes – 30 min incubation at 37°C	213
Figure 6.61 Complexation efficiency of PEGylated lipoplexes – 30 min incubation at 45°C	213
Figure 6.62 Complexation efficiency of Non-PEGylated lipoplexes	219
Figure 6.63 Complexation efficiency of PEGylated lipoplexes – 60 min incubation	220
Figure 6.64 Complexation efficiency of BHDSPE lipoplexes	220
Figure 6.65 Complexation efficiency of HDSPE lipoplexes	220
Figure 6.66 Complexation efficiency of BCDSPE lipoplexes.....	221
Figure 6.67 Complexation efficiency of CDSPE lipoplexes	221
Figure 6.68 Complexation efficiency of BADSPE lipoplexes	221

Figure 6.69 Complexation efficiency of ADSPE lipoplexes	222
Figure 6.70 TEM image of liposomes of BHDSPE lipoplexes	225
Figure 6.71 TEM image of liposomes of HDSPE lipoplexes.....	225
Figure 6.72 TEM image of liposomes of BCDSPE lipoplexes	225
Figure 6.73 TEM image of liposomes of CDSPE lipoplexes	226
Figure 6.74 TEM image of liposomes of BADSPE lipoplexes	226
Figure 6.75 TEM image of liposomes of ADSPE lipoplexes.....	226
Figure 7.1 Effect of electrolyte on particle size of the lipoplexes and non-PEGylated lipoplexes	234
Figure 7.2 Effect of electrolyte on particle size of the SA-DSPE, BHDSPE and HDSPE lipoplexes	235
Figure 7.3 Effect of electrolyte on particle size of the SA-DSPE, BCDSPE and CDSPE lipoplexes.....	235
Figure 7.4 Effect of electrolyte on particle size of the SA-DSPE, BADSPE and ADSPE lipoplexes.....	236
Figure 7.5 Effect of electrolyte on zeta potentials of the lipoplexes	237
Figure 7.6 Effect of electrolyte (2% NaCl) on complexation on efficiency of non- PEGylated and PEGylated lipoplexes at N/P ratio of 1.0 and 2.0 (200 ng pDNA/well)	240
Figure 7.7 Effect of electrolyte (2% NaCl) on complexation on efficiency of SA-DSPE, BHDSPE and HDSPE lipoplexes at N/P ratio of 2.0 (200 ng pDNA/well)	240
Figure 7.8 Effect of electrolyte (2% NaCl) on complexation on efficiency of SA-DSPE, BCDSPE and CDSPE lipoplexes at N/P ratio of 2.0 (200 ng pDNA/well)	241
Figure 7.9 Effect of electrolyte (2% NaCl) on complexation on efficiency of SA-DSPE, BADSPE and ADSPE lipoplexes at N/P ratio of 2.0 (200 ng pDNA/well)	241
Figure 7.10 Serum stability of naked pDNA	244
Figure 7.11 Serum stability of lipoplexes (SA-DSPE, BHDSPE and HDSPE) prepared at N/P ratio of 2 after incubation with serum	245
Figure 7.12 Serum stability of lipoplexes (BCDSPE and CDSPE) prepared at N/P ratio of 2 after incubation with serum	246
Figure 7.13 Serum stability of lipoplexes (BADSPE and ADSPE) prepared at N/P ratio of 2 after incubation with serum	246

Figure 8.1 Hemoglobin absorption spectra obtained with 0.1 mM SA-DSPE liposomes (pink), negative control (black) and positive control (green)	256
Figure 8.2 Haemolytic Potential of Lipoplexes	256
Figure 8.3 MTT Dye Reduction by Mitochondrial Reductase Enzyme of Viable Cells	258
Figure 8.4 Haemocytometer diagram indicating the 16 corners squares which should be used for counting.....	260
Figure 8.5 Cytotoxicity of liposomes prepared without and with DOPE (SA/DOPE, SA:HSPC:Chol liposomes and SA DSPE-nonPEGylated liposomes respectively) and PEGylated liposomes against DOTAP/DOPE liposomes.	264
Figure 8.6 Cytotoxicity of BHDSPE, BCDSPE and BADSPE liposomes against SADSPE liposomes and DOTAP/DOPE liposomes	265
Figure 8.7 Cytotoxicity of HDSPE, CDSPE and ADSPE liposomes against SA-DSPE liposomes and DOTAP/DOPE liposomes	265
Figure 8.8 Cytotoxicity of lipoplexes prepared with liposomes without and with DOPE (SA/DOPE, SA:HSPC:Chol liposomes and SA-DSPE nonPEGylated liposomes respectively) and PEGylated SA-DSPE liposomes against DOTAP/DOPE and lipofectamine-2000 lipoplexes.	266
Figure 8.9 Cytotoxicity of BHDSPE, BCDSPE and BADSPE lipoplexes against SA, DOTAP/DOPE and lipofectamine-2000 lipoplexes.....	267
Figure 8.10 Cytotoxicity of HDSPE, CDSPE and ADSPE lipoplexes against SA, DOTAP/DOPE and lipofectamine-2000 lipoplexes.....	267
Figure 8.11 %GFP expression observed after transfection with naked pDNA, lipoplexes of SA-DSPE and Modified DSPE lipoplexes. Untreated cells were taken as negative control while cells treated with lipoplexes of lipofectamine-2000 were taken as a reference control for comparison.	272
Figure 8.12 Overlay histograms of FACS analyses of cellular expression of eGFP by naked pDNA, lipoplexes of SA-DSPE and Modified DSPE lipoplexes. Untreated cells were taken as negative control while cells treated with lipoplexes of lipofectamine-2000 were taken as a reference control for comparison.	273

Figure 8.13 Confocal images of SA-DSPE, BHDSPE and HDSPE lipoplexes. Cellular expression is shown in comparison to that after transfection done with naked eGFP pDNA, lipoplexes of DOTAP/DOPE and Lipofectamine-2000.	276
Figure 8.14 Confocal images of GFP expression after transfection with BCDSPE and CDSPE lipoplexes. Cellular expression is shown in comparison to that with naked eGFP pDNA and lipoplexes of SA, DOTAP/DOPE and Lipofectamine-2000.	277
Figure 8.15 Confocal images of GFP expression after transfection with BADSPE and ADSPE lipoplexes. Cellular expression is shown in comparison to that with naked eGFP pDNA and lipoplexes of SA, DOTAP/DOPE and Lipofectamine-2000.	278
Figure 9.1 Complexation efficiency of lipoplexes before and after ligand conjugation	283
Figure 9.2 Effect of ligand conjugation on the cytotoxicity of the lipoplexes. Cytotoxicity of targeted and non-targeted lipoplexes are shown against DOTAP/DOPE lipoplexes.	285
Figure 9.3 %GFP expression observed after transfection with ligand conjugated and non-conjugated lipoplexes. Untreated cells were taken as negative control while cells treated with naked pDNA and lipoplexes of lipofectamine-2000 were taken as a reference control for comparison.	286
Figure 9.4 Confocal images of GFP expression after transfection with ligand conjugated and non-conjugated lipoplexes.	287
Figure 10.1 Histopathologic evaluation of aorta isolated from mice at different time points for various treatment groups (T0- initial time point; T-1M: at one month; T-2M: at 2 month)	305
Figure 10.2 The areas of atherosclerotic lesions in untreated (HFD) and treated (HDSPE lipoplexes and gHDSPE lipoplexes) animals (n = 4 in each group) are shown in the graph (overlapping dots are not shown) at end of 2 months. The line indicates the mean value.....	307