

## LIST OF FIGURES

Figure. 2.1	Methods for the preparation of mPEG- phosphatidyl ethanolamine conjugates	42
Figure 2.2	End group functionalized PEG lipids	44
Figure 2.3	Amphiphilic synthetic polymers used for steric protection of liposomes	45
Figure 3.1	Calibration plot for the estimation of phosphatidyl choline	123
Figure 3.2	Calibration plot for the estimation of Cholesterol	127
Figure 3.3	Absorptivity scan of cyclosporine in THF/Methanol	130
Figure 3.4	Calibration plot for the estimation of cyclosporine	130
Figure 3.5	Absorptivity scan of leuprolide acetate in 0.1N NaOH (50µg/ml)	133
Figure 3.6	Calibration curve for leuprolide acetate in 0.1N NaOH	134
Figure 3.7	Absorptivity of DNA in Tris buffer (20µgm/ml)	137
Figure 3.8	Calibration curve for DNA in Tris buffer	137
Figure 3.9	Absorptivity scan of leuprolide acetate in PBS (100µg/ml)	140
Figure 3.10	Calibration curve for leuprolide acetate in PBS	140
Figure 3.11	Absorptivity scan of DNA in PBS (20µg/ml)	142
Figure 3.12	Calibration curve for DNA in PBS	143
Figure 3.13	Calibration curve for mPEG5000-CC-PE	146
Figure 3.14	Calibration curve for mPEG2000-CC-PE	147
Figure 4.1	Flowchart for the preparation of charged liposomes containing cyclosporine	169
Figure 4.2	Flowchart for the preparation of conventional liposomes containing leuprolide acetate	180
Figure 4.3	The feed forward back-propagation network	183
Figure 4.4	Squared correlation coefficients ( $r^2$ ) for 27 formulations as a function of the number of hidden nodes using ANN	184
Figure 4.5	Contour plots of leuprolide acetate loaded liposome by $3^3$ factorial design (A) at -1 level of variable $X_3$ , (B) at 0 level of variable $X_3$ , (C) at 1 level of variable $X_3$	185
Figure 4.6	Flowchart for the preparation of conventional liposomes containing DNA	188
Figure 4.7	Results of electrolyte induced flocculation test on conventional liposomes containing Leuprolide acetate	191
Figure 4.8	Results of electrolyte induced flocculation test on conventional liposomes containing DNA	192
Figure 4.9	Ultraviolet absorptivity scan of cyanuric chloride (CC) in methanol	196
Figure 4.10	Ultraviolet absorptivity scan of methoxy polyethylene glycol 5000 (mPEG5000) in methanol (1mg/ml)	196

Figure 4.11	Ultraviolet absorptivity scan of methoxy polyethylene glycol 5000 activated with cyanuric chloride (mPEG5000-CC) in methanol (1mg/ml)	197
Figure 4.12	Mid infra-red spectrum of methoxy polyethylene glycol 5000 activated with cyanuric chloride (mPEG5000-CC)	198
Figure 4.13	<sup>13</sup> C-NMR spectrum of methoxy polyethylene glycol 5000 activated with cyanuric chloride (mPEG5000-CC)	199
Figure 4.14	Reaction scheme for the synthesis of methoxy polyethylene glycol 5000 activated with cyanuric chloride-phosphatidylethanolamine conjugate (mPEG5000-CC-PE)	200
Figure 4.15	Ultraviolet absorptivity scan of Phosphatidyl ethanolamine (PE) in methanol (1 mg/ml)	201
Figure 4.16	Ultraviolet absorptivity scan of methoxy polyethylene glycol 5000 activated with cyanuric chloride – phosphatidyl ethanolamine conjugate (mPEG5000-CC-PE) in methanol (5mg/ml)	201
Figure 4.17	Mid infra-red spectrum of methoxy polyethylene glycol 5000 activated with cyanuric chloride –phosphatidyl ethanolamine conjugate (mPEG5000-CC-PE)	202
Figure 4.18	<sup>13</sup> C-NMR spectrum of methoxy polyethylene glycol 5000 activated with cyanuric chloride –phosphatidyl ethanolamine conjugate (mPEG5000-CC-PE)	203
Figure 4.19	Ultra violet absorptivity scan of methoxy polyethylene glycol 2000 in methanol (1mg/ml)	206
Figure 4.20	Ultraviolet absorptivity scan of methoxy polyethylene glycol 2000 activated with cyanuric chloride (mPEG2000-CC) in methanol (1mg/ml)	206
Figure 4.21	Mid infra-red spectrum of methoxy polyethylene glycol 2000 activated with cyanuric chloride (mPEG2000-CC) in methanol	207
Figure 4.22	Reaction scheme of synthesis of methoxy polyethylene glycol 2000 activated with cyanuric chloride-phosphatidyl ethanolamine conjugate (mPEG2000-CC-PE)	208
Figure 4.23	Ultraviolet absorptivity scan of methoxy polyethylene glycol 2000 coupled with cyanuric chloride -phosphatidyl ethanolamine conjugate (mPEG2000-CC-PE) in methanol (1mg/ml)	209
Figure 4.24	Mid infra-red spectrum of methoxy polyethylene glycol 2000 activated with cyanuric chloride –phosphatidyl ethanolamine conjugate (mPEG2000-CC-PE)	210
Figure 4.25	<sup>13</sup> C-NMR spectrum of methoxy polyethylene glycol 2000 activated with cyanuric chloride – phosphatidyl ethanolamine conjugate (mPEG2000-CC-PE)	211
Figure 4.26	Flowchart for the preparation of sterically stabilized liposomes containing Leuprolide acetate	212

Figure 4.27	Optimization of mPEG-5000-CC-PE concentration, required for steric stabilization of leuprolide acetate containing liposomes, using electrolyte induced flocculation test	213
Figure 4.28	Optimization of mPEG-5000-CC-PE concentration, required for steric stabilization of leuprolide acetate containing liposomes, using electrolyte induced flocculation test	214
Figure 4.29	Flowchart for the preparation of sterically stabilized liposomes containing DNA	215
Figure 4.30	Optimization of mPEG5000-CC-PE concentration, required for steric stabilization of DNA containing liposomes, using electrolyte induced flocculation test	216
Figure 4.31	Optimization of mPEG2000-CC-PE concentration, required for steric stabilization of DNA containing liposomes, using electrolyte induced flocculation test	217
Figure 4.32	Optimization of mPEG2000-CC-PE concentration, required for steric stabilization of DNA containing liposomes, using electrolyte induced flocculation test	218
Figure 5.1	Photomicrograph of conventional liposomes containing cyclosporine	244
Figure 5.2	Photomicrograph of positive charged liposomes containing cyclosporine	244
Figure 5.3	Photomicrograph of negative charged liposomes containing cyclosporine	245
Figure 5.4	Photomicrograph of conventional liposomes containing leuprolide acetate	245
Figure 5.5	Photomicrograph of liposomes containing leuprolide acetate sterically stabilized using methoxy polyethylene glycol 5000 -activated with cyanuric chloride-phosphatidylethanolamine conjugate	246
Figure 5.6	Photomicrograph of liposomes containing leuprolide acetate sterically stabilized using methoxy polyethylene glycol 2000 -activated with cyanuric chloride-phosphatidylethanolamine conjugate	246
Figure 5.7	Photomicrograph of conventional liposomes containing DNA	247
Figure 5.8	Photomicrograph of liposomes containing DNA sterically stabilized using methoxy polyethylene glycol 5000 - activated with cyanuric chloride -phosphatidyl ethanolamine conjugate	247
Figure 5.9	Photomicrograph of liposomes containing DNA sterically stabilized using methoxy polyethylene glycol 2000- activated with cyanuric chloride -phosphatidyl ethanolamine conjugate	248

Figure 5.10	Photomicrograph of cationic liposomes containing DNA sterically stabilized using methoxy polyethylene glycol 2000-activated with cyanuric chloride-phosphatidylethanolamine conjugate	248
Figure 5.11	Scanning electron micrograph of conventional liposomes containing cyclosporine	249
Figure 5.12	Scanning electron micrograph of conventional liposomes containing leuprolide acetate	249
Figure 5.13	Scanning electron micrograph of liposomes containing leuprolide acetate sterically stabilized using mPEG5000-CC-PE	249
Figure 5.14	Scanning electron micrograph of conventional liposomes containing DNA	250
Figure 5.15	Scanning electron micrograph of conventional liposomes containing DNA sterically stabilized using mPEG2000-CC-PE	250
Figure 5.16	DSC thermogram of conventional liposomes containing cyclosporine	251
Figure 5.17	DSC thermogram of positive charged liposomes containing cyclosporine	252
Figure 5.18	DSC thermogram of conventional liposomes containing leuprolide acetate	253
Figure 5.19	DSC thermogram of liposomes containing leuprolide acetate sterically stabilized using methoxy polyethylene glycol 2000 -activated with cyanuric chloride – phosphatidyl ethanolamine conjugate (mPEG2000-CC-PE)	254
Figure 5.20	DSC thermogram of conventional liposomes containing DNA	255
Figure 5.21	DSC thermogram of liposomes containing DNA sterically stabilized using methoxy polyethylene glycol 5000 – activated with cyanuric chloride –phosphatidyl ethanolamine conjugate (mPEG5000-CC-PE)	256
Figure 6.1	<i>In vitro</i> drug release studies of neutral liposomes containing cyclosporine	274
Figure 6.2	<i>In vitro</i> drug release studies of positive charged liposomes containing cyclosporine	274
Figure 6.3	<i>In vitro</i> drug release studies of negative charged liposomes containing cyclosporine	275
Figure 6.4	<i>In vitro</i> drug release studies of conventional liposomes containing leuprolide acetate	275
Figure 6.5	<i>In vitro</i> drug release studies of sterically stabilized liposomes (SLL5000) containing leuprolide acetate	276
Figure 6.6	<i>In vitro</i> drug release studies of sterically stabilized liposomes (SLL2000) containing leuprolide acetate	276

Figure 6.7	<i>In vitro</i> drug release studies of conventional liposomes containing DNA	277
Figure 6.8	<i>In vitro</i> drug release studies of sterically stabilized liposomes (SDL5000) containing DNA	277
Figure 6.9	<i>In vitro</i> drug release studies of sterically stabilized liposomes (SDL2000) containing DNA	278
Figure 6.10	<i>In vitro</i> drug release studies of cationic sterically stabilized liposomes (CSDL2000) containing DNA	278
Figure 7.1	Effect of sucrose and lactose on the % retention of CsA loaded positive charged liposomes	302
Figure 7.2	Effect of sucrose and trehalose on the % retention of leuprolide acetate loaded conventional liposomes	302
Figure 8.1	Flow cytometric DNA profiles of PI stained splenocytes treated with different concentrations of cyclosporine <i>in vitro</i> along with the percentage apoptosis of splenocytes shown below each DNA histograms	314
Figure 8.2	Concentration dependent apoptosis of cyclosporine on mouse splenocytes	315
Figure 8.3	Flow cytometric DNA profiles of PI stained splenocytes treated with CsA and its liposomal preparation studied by the flow cytometric analysis of DNA content, expressed as the number of apoptotic cells.	316
Figure 8.4	Induction of apoptosis in Splenic lymphocytes at 24 h after incubation with CsA and its liposomal preparation studied by the flow cytometric analysis of DNA content, expressed as the percentage of the number of apoptotic cells.	317
Figure 9.1	Time dependant <i>in vitro</i> cytotoxicity study of leuprolide acetate	327
Figure 9.2	<i>In vitro</i> cytotoxicity study of leuprolide acetate and its liposomes	328
Figure 10.1	Effect of pH on radiolabeling efficiency of cyclosporine and its liposomal formulations	339
Figure 10.2	Effect of incubation time on radiolabeling efficiency of cyclosporine and its liposomal formulations	340
Figure 10.3	DTPA challenging test of cyclosporine and its liposomal formulations	343
Figure 10.4	Effect of pH on radiolabeling efficiency of leuprolide and its liposomal formulations	345
Figure 10.5	Effect of incubation time on radiolabeling efficiency of leuprolide and its liposomal formulations	346
Figure 10.6	DTPA challenging test of leuprolide and its liposomal formulations	349
Figure 10.7	Effect of pH on radiolabeling efficiency of DNA and its liposomal formulations	351

Figure 10.9	DTPA challenging test of DNA and its liposomal formulations	355
Figure 11.1	Blood kinetic study of $^{99m}\text{Tc}$ -cyclosporine, $^{99m}\text{Tc}$ -CPL, $^{99m}\text{Tc}$ -CNL, and $^{99m}\text{Tc}$ -CL in rabbits.	368
Figure 11.2	Results of the blood kinetic data of leuprolide acetate and its liposomes	371
Figure 11.3	Results of the blood kinetic data of DNA and its liposomes	374
Figure 11.4	Bone/RES ratio of cyclosporine and its liposomes	380
Figure 11.5	Blood/RES ratio of cyclosporine and its liposomes	380
Figure 11.6	Tumor/RES ratio of Leuprolide acetate and its liposomes	385
Figure 11.7	Tissue uptake ratio of Leuprolide acetate and its liposomes (24 h post injection)	385
Figure 11.8	Compartmental distribution of leuprolide acetate loaded liposomes versus time after 1 h, 4 h, 24 h, and 48 h injection	386
Figure 11.9	Tissue uptake ratio of DNA and its liposomes	392
Figure 11.10	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -CsA.	395
Figure 11.11	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -CPL.	395
Figure 11.12	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -CL.	396
Figure 11.13	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -CNL.	396
Figure 11.14	Gamma scintigraphic image of leuprolide acetate injected mice bearing EAT in the left thigh	397
Figure 11.15	Gamma scintigraphic image of LL injected mice bearing EAT in the left thigh	397
Figure 11.16	Gamma scintigraphic image of SLL5000 injected mice bearing EAT in the left thigh	398
Figure 11.17	Gamma scintigraphic image of SLL2000 injected mice bearing EAT in the left thigh	398
Figure 11.18	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -DNA.	399
Figure 11.19	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -DL.	399
Figure 11.20	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -SDL5000.	400
Figure 11.21	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -SDL2000.	400
Figure 11.22	Gamma camera image of a rabbit at 4h after I.V. administration of $^{99m}\text{Tc}$ -CSDL2000.	401

Figure 12.1	Kidney from control male showing normal morphology of epithelial cells located in the outer stripe of the outer medulla	421
Figure 12.2	Kidney from male rat treated with 20mg/kg/day cyclosporine for 15 days	421
Figure 12.3	Kidney from male rat treated with 20mg/kg/day CPL for 15 days	422
Figure 12.4	Kidney from male rat treated with 20mg/kg/day CNL for 15 days	422