

List of Figures

Figure 1-1 Tissue differentiation in normal lung, adenocarcinoma and squamous cell carcinoma. Reference: S.S.G hospital, Vadodara.	2
Figure 1-2 RNAi mechanism (29).....	6
Figure 2-1 Pathogenesis of lung cancer.....	18
Figure 2-2 Demographic representation of Docetaxel mechanism of action	22
Figure 2-3 Cellular factors responsible for drug resistance	23
Figure 2-4 Physiological factors responsible for drug resistance	24
Figure 2-5 Gene silencing mechanism through RNA interference technology (RNAi) (32).....	26
Figure 2-6 Difference between gene silencing effect of siRNA and shRNA	28
Figure 2-7 Two-step method.....	33
Figure 2-8 Single step method	34
Figure 2-9 Seven-phase ladder for optimizing drug delivery systems	39
Figure 2-10 Different types of responses as functions of factor settings; (a) linear; (b) quadratic; (c) cubic.....	41
Figure 2-11 Types of Dry Powder Inhalers	43
Figure 2-12 Particle size distribution and cascade impactor	47
Figure 3-1 Docetaxel $\lambda_{\max}$ determination.....	67
Figure 3-2 Calibration plot of Docetaxel in Acetonitrile by UV-VIS spectrophotometry	68
Figure 3-3 Calibration plot of Docetaxel in Acetonitrile.....	68
Figure 3-4 Overlay Calibration plot of Docetaxel in Phosphate buffer 7.4by UV spectrophotometry.....	70
Figure 3-5 Calibration plot of Docetaxel in Phosphate buffer 7.4.....	71
Figure 3-6 Overlay Calibration plot of Docetaxel in Phosphate buffer 6.6 by UV spectrophotometry.....	73
Figure 3-7 Calibration plot of Docetaxel in Phosphate buffer 6.6.....	73
Figure 3-8 Overlay Calibration plot of Docetaxel in Acetate buffer 5.5by UV spectrophotometry.....	75
Figure 3-9 Calibration plot of Docetaxel in Acetate buffer 5.5.....	76
Figure 3-10 Overlay Chromatogram of Docetaxel by RP-HPLC	78
Figure 3-11 Overlay Chromatogram of Docetaxel by RP-HPLC	78

Figure 3-12 Calibration plot of Docetaxel by RP-HPLC	79
Figure 3-13 Overlay Chromatogram of Docetaxel in rat plasma by RP-HPLC	81
Figure 3-14 Overlay Chromatogram of Docetaxel in rat plasma by RP-HPLC .	81
Figure 3-15 Calibration plot of Docetaxel by RP-HPLC in rat plasma.....	82
Figure 3-16 Overlay Chromatogram of Docetaxel in rat Lung homogenate by RP-HPLC.....	84
Figure 3-17 Overlay Chromatogram of Docetaxel in rat Lung homogenate by RP-HPLC.....	84
Figure 3-18 Calibration plot of Docetaxel by RP-HPLC in rat Lung homogenate.....	85
Figure 3-19 Overlay Calibration plot of total phospholipids mixture in chloroform..	87
Figure 3-20 Calibration plot of total phospholipids mixture in chloroform	87
Figure 3-21 Graph of analytical interference study of Docetaxel and Formulation excipients.....	89
Figure 3-22 UV absorption profile of isolated pDNA.....	90
Figure 3-23 Correlation of actual concentration of pDNA vs observed concentration	91
Figure 3-24 Determination of quantifiable range of pDNA	92
Figure 3-25 Band densities at 200 ng Lane 1 to Lane 6: 200 ng pDNA.	93
Figure 3-26 Calibration plot of DNA gel retardation.	94
Figure 4-1 DSC Thermogram of Docetaxel.....	99
Figure 4-2 a) IR spectra of Docetaxel sample Vs. b) IR spectra of standard Docetaxel	100
Figure 4-3 DSC thermogram for Compatibility study.....	101
Figure 4-4 Compatibility study by infrared spectroscopy	102
Figure 4-5 Stability of shRNA pDNA stock after repetitive sampling	103
Figure 4-6 Effect of temperature and pH on shRNA pDNA	104
Figure 5-1 Particle size distribution of L/P ratio 35.....	112
Figure 5-2 Particle size distribution of L/P ratio 25	112
Figure 5-3 Pareto Chart for the selected factorial model of Encapsulation efficiency	119
Figure 5-4 Pareto Chart for a selected factorial model of particle size	121
Figure 5-5 Normal plot of residuals for encapsulation efficiency	125
Figure 5-6 Residual vs. Predicted plot for encapsulation efficiency.....	126
Figure 5-7 Residual vs. Run plot for encapsulation efficiency.....	127

Figure 5-8 Predicted vs. Actual plot for the encapsulation efficiency	128
Figure 5-9 Box-Cox plot for power transformation for the encapsulation efficiency	129
Figure 5-10 Residual vs. Individual factor plots.....	129
Figure 5-11 Piepel's plot.....	130
Figure 5-12 Effects of various factors on DTX entrapment by 3D surface curve	131
Figure 5-13 Normal plot of residuals for particle size.....	134
Figure 5-14 Residual vs. Predicted plot for particle size	135
Figure 5-15 Residual vs. Run plot for particle size	136
Figure 5-16 Predicted vs. Actual plot for the particle size	137
Figure 5-17 Box-Cox plot for Power transformation for the particle size	138
Figure 5-18 Residual vs. Individual factor plots.....	138
Figure 5-19 Piepel's plot for particle size.....	139
Figure 5-20 Effects of various factors on PLHNCs particle size by 3D surface curve	140
Figure 5-21 Desirability plot.....	141
Figure 5-22 Size and PDI of optimized PLHNCs batch.....	148
Figure 5-23 Zeta potential of optimized PLHNCs batch.....	149
Figure 5-24 SEM images of optimized batch of PLHNCs	150
Figure 5-25 TEM images of optimized batch of PLHNCs	150
Figure 5-26 Docetaxel drug release pattern in different release media	151
Figure 5-27 Release of shRNA pDNA from PLHNCs.....	152
Figure 5-28 In-Vitro Release model of Docetaxel	152
Figure 5-29 In-Vitro Release model of shRNA pDNA	153
Figure 5-30 Graph of residual solvent (acetonitrile) estimation	154
Figure 5-31 A) 3D image of PLHNC surface B) Z-surface histogram for estimation of PEGylation layer.....	155
Figure 6-1 A) Cryo-TEM of lyophilized PLHNCs (B) freeze fracture TEM of lyophilized PLHNCs.	166
Figure 6-2 Weight fraction of Docetaxel at different stages and plates in Anderson cascade impactor.....	168
Figure 6-3 SEM images of developed PLHNCs	169
Figure 6-4 Comparison of PXRD graph of Docetaxel and PLHNC DPI formulation	170

Figure 6-5 DSC graph comparison of Docetaxel and D-PLHNCs DPI	171
Figure 6-6 IR graph comparison of Docetaxel and developed D-PLHNCs DPI	171
Figure 6-7 Integrity of shRNA pDNA.....	172
Figure 7-1 MTT Dye Reduction by Mitochondrial Reductase Enzyme of Viable Cells	178
Figure 7-2 Change in % viability of the parent A549 cells and DR-A549 cells on treatment with Docetaxel solution.	190
Figure 7-3 Cytotoxicity studies on DR-A549 cell line.....	191
Figure 7-4 Cell migration assay	192
Figure 7-5 Live confocal microscopy of cellular uptake at different time intervals for naked shRNA pDNA, sh-PLHNCs, FA-sh-PLHNCs, and Lipofectamine 2000-shRNA pDNA complex.	194
Figure 7-6 Quantification of live confocal images at the period of 5- min, 10-min, 15-min, 20-min, and 25 min interval with various treatment using ImageJ software	195
Figure 7-7 Quantitative uptake using FACS histogram	195
Figure 7-8 Estimation of P-gp inhibition using Rh123.	196
Figure 7-9 A) Qualitative measurement of endocytic uptake using Confocal images B) Quantitative measurement of fluorescence intensity using a fluorescent microplate reader	198
Figure 7-10 Detection of caspase-3 level in DR-A549 cell line using CASP-3-C kit, treated with different docetaxel and hybrid formulation and incubated for 6 and 12 h.....	199
Figure 7-11 Apoptotic analysis of DR-A549 cells in a time-dependent manner with different treatment.....	200
Figure 7-12 Apoptosis cell analysis of DR-A549 cell line after different Docetaxel formulation upon 24h incubation.....	201
Figure 7-13 Distribution of cell cycle in DR-A549 cells after 24 h incubation.....	202
Figure 7-14 Cell cycle histogram after 24 h incubation with different Docetaxel formulation.	202
Figure 7-15 Impact on MDR1 mRNA upon treatment with various concentrations of ABCB1 shRNA in DR-A549 cells.....	203
Figure 8-1 Histogram images of Sprague Dawley rat lungs after intratracheal instillation	213
Figure 8-2 Concentration of Docetaxel in BAL and LH.	214
Figure 8-3 Enzyme activity and L/B ratio	215

Figure 8-4 Metabolism of Docetaxel solution, D-PLHNCs, and FA-D-PLHNCs in Sprague Dawley lung and liver microsomes	216
Figure 8-5 Haemolysis by UV spectroscopy	217
Figure 8-6 Hemolysis by Optical method.....	217