

## INDEX

<b>CONTENT</b>	<b>Page No.</b>
<b>1. Introduction</b>	02
1.1 Research Envisaged	09
1.2 Proposed Plan of work	09
1.3 References	11
<b>2. Review of Literature</b>	14
2.1 Factors affecting pulmonary drug delivery	16
2.1.1 Mechanisms of particle deposition in airways	16
2.1.2 Physiological factors affecting particle deposition in airways	17
2.1.2.1 Lung Morphology	17
2.1.3 Pharmaceutical factors affecting aerosol deposition	19
2.2 Fate of particles in the airways	21
2.2.1 Mucus barrier	21
2.2.2 Mucociliary Clearance	21
2.2.3 Alveolar Clearance	22
2.3 Factors affecting the absorption and metabolism of drugs in the airways	22
2.3.1 Area	22
2.3.2 Absorption Barrier Thickness	23
2.3.3 Blood Supply	23
2.3.4 Membrane permeability	23
2.3.5 Enzymatic activity	24
2.4 Merits and Demerits of pulmonary drug delivery	25
2.4.1 Locally acting drugs	25

2.4.2 Merits and demerits of systemically acting drugs	25
2.5 Lung cancer	26
2.6 Drug Profile	31
2.6.1 Etoposide	31
2.6.1.1 Physico Chemical aspects	32
2.6.1.2 Pharmacological aspects	32
2.6.1.3 Dose and Route of administration	34
2.6.1.4 Marketed preparation	35
2.6.2 Docetaxel	35
2.6.2.1 Physico chemical aspects	36
2.6.2.2 Pharmacology	36
2.6.2.3 Dose and Route of administration	38
2.7 Liposomes	38
2.7.1 Composition of liposomes	39
2.7.1.1 Phospholipids	40
2.7.1.2 Sterols	41
2.7.1.3 Non structural components	41
2.7.2 Types of liposomes	41
2.7.3 Methods of preparation of liposomes	42
2.7.3.1 Passive loading techniques	42
2.7.3.1.1 Thin film hydration using hand shaking (MLVs) and non shaking	43
Methods	
2.7.4 Remote (Active) loading	50

2.8 Characterization of liposomes	.52
2.8.1 Vesicle shape and lamellarity	52
2.8.2 Vesicle size and distribution	53
2.8.2.1 Microscopic Techniques	53
2.8.3 Surface charge	55
2.8.4 Encapsulation Efficiency	55
2.8.5 Trapped Volume	56
2.8.6 Phase Response and transitional behaviour	56
2.8.7 Stability of liposomes	56
2.9 Drug Delivery to tumours	58
2.9.1 Development of tumour	58
2.9.2 Molecular targets for tumour therapy	59
2.9.2.1 Targeted drug delivery via folate receptor	61
2.9.2.2 Hyaluronic acid as a ligand	61
2.10 Dry Powder Inhalers	64
2.10.1 Powder and Aerosol physico chemical characterization	66
2.10.1.1 Moisture content and Hygroscopicity	67
2.10.1.2 Particle size	67
2.10.1.3 Aerodynamic Diameter and Dynamic shape factor	68
2.10.1.4 Fine Particle Fraction	69
2.10.1.5 Polydispersity	69
2.11 Formulation of DPIs	72
2.11.1 Excipients	72

2.12 Pharmaceutical Processing	74
2.13 A549 profile	76
2.14 References	80
<b>3. Analytical Methods</b>	<b>88</b>
3.1 Materials and Methods	90
3.2 Chemical Analysis	91
3.2.1 Estimation of Etoposide (ETP) by UV spectrophotometric method	91
3.2.1.1 Estimation of Etoposide (ETP) by HPLC	92
3.2.1.2 Estimation of Etoposide in non grafted and grafted liposomes	95
3.2.1.3 Estimation of Etoposide in diffusion medium	95
3.2.1.4 Estimation of Etoposide in biological samples (Cell lysates)	95
3.2.2 Estimation of Docetaxel (DOC)	97
3.2.2.1 Estimation of Docetaxel in solution by UV spectrophotometry	97
3.2.2.1.1 Estimation of Docetaxel by HPLC	98
3.2.3 Estimation of Docetaxel in non grafted and grafted liposomes	101
3.2.3.1 Estimation of DOC in diffusion medium	101
3.2.3.2 Estimation of DOC in biological samples (Cell lysates)	101
3.3 Determination of protein in cell lysates by BCA method	103
3.4 Spectrophotometric determination of Hyaluronic acid (HA)	106
3.5 Physical Characterization	
3.5.1 Determination of particle size and Polydispersity	107
3.5.2 Determination of zeta potential	107
3.5.3 Morphological characterization	107

3.5.4 Differential Scanning Calorimetry	108
3.5.5 Fourier Transform Infra Red (FTIR) Analysis	108
3.6 Discussion	108
3.7 References	110
<b>4. Preparation and Optimization of Liposomes</b>	<b>112</b>
4.1 Materials and methods	116
4.2 Preparation of liposomes of Etoposide (ETPLIP) and Docetaxel (DOCLIP)	118
4.2.1 Preparation of ETP liposomes by TFH method	118
4.2.2 Preparation of DOC liposomes by TFH method	120
4.2.3 Preparation of 6-coumarin loaded liposomes by TFH method	121
4.3 Optimization of liposomal formulation using $2^3$ factorial design	122
4.3.1 Optimization of formulation components for drug (ETP/DOC) loaded liposomes	122
4.3.1.1 Contour plots	131
4.3.1.2 Check point Analysis	134
4.4 Particle size reduction and separation of unentrapped drug	135
4.5 Characterization of liposomes	136
4.5.1 Particle Size Measurement	136
4.5.2 Zeta potential Determination	136
4.5.3 Percentage Drug Entrapment	136
4.6 Discussion	137
4.7 References	142

<b>5. Preparation and Optimization of ligand (Hyaluronic acid-HA) grafted liposomes</b>	<b>147</b>
5.1 Materials and methods	151
5.1.1 Preparation and optimization of HA grafted Etoposide liposomes (HAETPLIP) and Docetaxel liposomes	152
5.1.2 Estimation and optimization of concentration of coupling agent (EDC)	155
5.1.3 Spectrophotometric determination of HA	160
5.1.4 Fourier Transform Infra red spectroscopy	161
5.1.5 Preparation and optimization of HA grafted coumarin loaded liposomes	166
5.2 Discussion	167
5.3 Conclusion	171
5.4 References	173
<b>6. Characterization of drug loaded (HA grafted and non grafted) liposomes</b>	<b>176</b>
6.1 Equipments	178
6.2 Physical Characterization	179
6.2.1 Particle Size Measurement	180
6.2.2 Zeta potential Determination	180
6.2.3 Percentage Drug Entrapment	180
6.3 Characterization of 6 coumarin loaded liposomes	181
6.4 Drug Excipient Interaction by DSC	182
6.5 In vitro drug release studies for non grafted and HA grafted liposomes	187
6.6 Discussion	192
6.7 References	196

<b>7. Preparation and optimization of Dry Powder Inhalers (DPIs) of HA grafted liposomes</b>	<b>197</b>
7.1 Materials and methods	203
7.2 Freeze Drying or lyophilization of HA grafted Etoposide liposomes	204
7.3 Freeze Drying or lyophilization of HA grafted Docetaxel liposomes	206
7.4 Characterization of liposomal DPI	208
7.4.1 Angle of repose	208
7.4.2 Compressibility Index	209
7.4.3 Tapped Density	209
7.4.4 Residual Moisture Content	209
7.4.5 Particle Size Determination	210
7.5 Characterization of aerosol performance	210
7.6 Surface Morphology assessment of DPIs	213
7.7 In vitro drug release studies	215
7.8 Discussion	219
7.9 References	223
<b>8. Stability studies of DPIs of HA grafted liposomal Etoposide and Docetaxel</b>	<b>227</b>
8.1 Methodology	229
8.2 Discussion	239
8.3 References	242
<b>9. Cell Line studies</b>	<b>243</b>
9.1 Materials and methods	248
9.2 Methodology	250

9.2.1 In vitro cell uptake studies	250
9.2.1.1 Preparation of 6-coumarin loaded liposomes	250
9.2.1.2 Cell Uptake studies	251
9.2.2 In vitro cytotoxicity studies by MTT assay	257
9.2.3 Pharmacokinetic (Intracellular Drug Concentration) Assessment	259
9.2.3.1 Effect of temperature and time of in vitro cell uptake	262
9.2.4 Cell Cycle Analysis by Flow Cytometry	264
9.3 Discussion	267
9.4 References	273
<b>10. Summary and Conclusion</b>	<b>277</b>
Summary	278
Conclusion	293
<b>List of Papers published/presented</b>	<b>295</b>