LIST OF TABLES

Table 2.1: Tumor marker associated with OC. 23

Table 2.2: Pharmaceutical derivatives of β-cyclodextrins 30

Table 2.3: Examples of Cyclodextrin-enhanced solubility and dissolution..... 30

Table 2.4: Barrier faced and strategies to enhance efficacy of nano-formulations..... 35

Table 2.5: Nanocarrier based active and passive targeting systems for OC under clinical trials. 39

Table 2.6: Antibody/Antibody fragment conjugated nanocarriers evaluated in preclinical studies for treatment of ovarian cancer 50

Table 2.7:Dosage regimen in treatment of various stages of Ovarian, Breast and Non-small cell lung cancer 56

Table 3.1 Calibration curve values of Paclitaxel in methanol at λmax 227 nm..... 81

Table 3.2 Parameters for Estimation of PTX by RP-HPLC method..... 82

Table 3.3 Intraday precision and accuracy data for estimation of Paclitaxel by RP-HPLC..... 82

Table 3.4 Interday precision and accuracy data for estimation of Paxlitaxel by RP-HPLC..... 83

Table 3.5 Calibration curve values of total phospholipid mixture in chloroform at λmax 472 nm 84

Table 3.6 Parameters for Estimation of total phospholipid mixture by Stewart method 84

Table 3.7 Calibration curves values of BSA at λmax 562 nm 85

Table 3.8 Parameters for Estimation of protein by BCA protein estimation method . 86

Table 3.9 Calibration curve values of BSA at λmax 595 nm..... 86

Table 3.10 Parameters for Estimation of protein by Bradford's method 87

Table 3.11 Calibration curve values of cysteine at λmax 412 nm 88

Table 3.12 Parameters for Estimation of cysteine by Ellman's assay 89

Table 3.13 Calibration data for Coumarin 6 in Chloroform:Methanol (1:1) 89

Table 3.14 RP-HPLC calibration curve values of paclitaxel in plasma at λmax at 227 nm..... 91

Table 3.15 Parameters for Estimation of PTX in plasma by RP-HPLC method. 92

LIST OF TABLES

Table 3.16 Intraday precision and accuracy data for estimation of Paclitaxel in plasma by RP-HPLC 92

Table 3.17 Interday precision and accuracy data for estimation of Paxlitaxel in plasma by RP-HPLC 93

Table 4.1 Abbreviated names of CDs and their derivatives..... 97

Table 4.2 PTX solubility enhancement by CDs..... 97

Table 5.1 Various variables and responses involved in optimization..... 127

Table 5.2 Coded and actual levels of HSPC, EPC and Chol used in optimization..... 127

Table 5.3 Lyophilization cycle..... 136

Table 5.4Stability Testing Conditions for Drug Product Intended for Storage in Refrigerator as per ICH Guideline Q1A(R2). 138

Table 5.5 Design Matrix for PEGylated Paclitaxel Loaded Liposome Optimization..... 140

Table 5.6 Summary of ANOVA results for Different Models..... 141

Table 5.7 ANOVA for Quadratic Mixture Model..... 142

Table 5.8 Summary of ANOVA results for Quadratic Mixture Model 143

Table 5.9 Summary of ANOVA results for Different Models..... 148

Table 5.10 ANOVA Table 6.for Quadratic Mixture Model 148

Table 5.11 Summary of ANOVA results for Quadratic Mixture Model 149

Table 5.12 Constraints Applied for Selection of Optimized Batch..... 154

Table 5.13 Optimized Batch Parameters Based on Desirability 155

Table 5.14 Predicted Responses of the Optimized Batch 156

Table 5.15 Experimental Confirmation of the Predicted Responses 156

Table 5.16 Optimization of process variables..... 157

Table 5.17 Effect of PEGylation..... 158

Table 5.18 Comparison of PEGylated liposomes prepared using individual and combination of lipid 159

Table 5.19 Impact of electrolyte on optical clarity of liposomes..... 171

Table 5.20 Effect of electrolyte on Particle size of liposomes..... 171

Table 5.21 % Liposomes Recovery..... 172

LIST OF TABLES

Table 5.22 Particle size and Zeta potential of liposomes before and after incubation with FBS 173

Table 5.23 Amount of serum proteins associated with liposomes 174

Table 5.24 Initial % CF latency of liposomes with PTX after incubation with serum at 37°C 175

Table 5.25 % retention of encapsulated CF after incubation with serum proteins at 37°C 175

Table 5.26 Effect of lyophilization on particle size and zeta potential of PLs and DLPLs 178

Table 5.27 Reconstitution time and water content of lyophilized liposomes 178

Table 5.28 Stability Testing Data of DLPLs (Suspension form) 179

Table 5.29 Stability Testing Data of DLPLs (Lyophilized form after reconstitution) 180

Table 6.1 Estimation of functionalization 199

Table 6.2 Composition of resolving gel. 205

Table 6.3 Composition of stacking gel 205

Table 6.4 The characteristic peaks exhibited by the functionalizing lipid. 208

Table 6.5 Ellman's assay: Determination of reacted thiol concentration 209

Table 6.6 Characterization of ILs 213

Table 7.1 Various treatments to cells for determination immunoreactivity of Fab′ fragments and Immunoliposomes 228

Table 7.2 Comparison of % relative mean fluorescence intensity of Caov3 cells treated with different concentration of Fab′ fragment and ILs to determine immunoreactivity towards FSHR. 242

Table 7.3 Percentage of cell death after treatment of Caov3 cells with various formuations at equimolar concentration 253

Table 8.1: The pharmacokinetic parameters of PTX after intravenous administration of Taxol®, DLPLs and ILs at 5 mg/kg PTX in SD rats (mean±S.D., n=6). 277

Table 8.2: Acute toxicity study results for DLPLs vs Taxol® 279

LIST OF FIGURES

Figure 2-1: Subtypes of Ovarian cancer tumors 21

Figure 2-2: Histologic subtypes of epithelial ovarian carcinoma and associated mutations/molecular aberrations 22

Figure 2-3: Liposomes as drug delivery carriers 28

Figure 2-4 Types of Cyclodextrins 30

Figure 2-5: Schematic representation of targeting approach and mechanisms by which nanocarriers (circles) can deliver drugs to tumours. 37

Figure 2-6: Receptor mediated endocytosis of nanocarrier. 38

Figure 2-7 FSHR 42

Figure 2-8: "Progress in the generation of monoclonal antibodies from murine to human and various antibody fragments. 48

Figure 2-9: Structure of Immunoliposomes 52

Figure 2-10: Mechanism of action of Paclitaxel 54

Figure 3.1: Principe and Reaction of BCA protein estimation method 75

Figure 3.2 Principle of Bradford Assay 77

Figure 3.3 Reduction of Ellman's Reagent 78

Figure 3.4 RP-HPLC chromatogram overlay of Paclitaxel in methanol at λmax 227 nm. 81

Figure 3.5 RP-HPLC calibration curve of paclitaxel in methanol at λmax 227 nm. .. 82

Figure 3.6 UV absorbance scans of total phospholipid mixture in chloroform at λmax 472 nm. 83

Figure 3.7 Calibration curve of total phospholipid mixture in chloroform at λmax 472 nm. 84

Figure 3.8 Calibration curve of BSA at λmax 562 nm 85

Figure 3.9 Calibration curve of BSA at λmax 595 nm 87

Figure 3.10 UV absorbance scans of cysteine at λmax 412 nm 88

Figure 3.11 Calibration curve of cysteine at λmax 412 nm 88

Figure 3.12 Calibration curve of Coumarin 6 in Chloroform:Methanol (1:1) 90

Figure 3.13 Spectra of different concentration of Coumarin 6 in Chloroform:Methanol (1:1) 90

LIST OF FIGURES

Figure 3.14 RP-HPLC chromatogram overlay of paclitaxel in plasma at λmax at 227 nm. 91

Figure 3.15 RP-HPLC calibration curve of paclitaxel in plasma at λmax at 227 nm. 92

Figure 4.1 General structure of CDs 96

Figure 4.2 Solubility of paclitaxel in A. HPβCD and B. HEβCD (C) Using the method for type B complexes, wherein above saturation solubility, precipitation is observed, solubility data for panels A and B were obtained 98

Figure 4.3 Entrapment efficiency and solubility of PTX ICs at various molar ratio. 105

Figure 4.4 DSC thermograms of PTX, DMβCD and PTX-DMβCD inclusion complex. 106

Figure 4.5 FTIR spectra of (A) DMβCD, (B) PTXD-β-CD ICs and (C) PTX. 107

Figure 4.6 X-ray diffraction patterns of PTX, DMβCD and PTXD-β-CD ICs..... 108

Figure 4.7 SEM images PTX-DMβCD ICs (left) and PTX (right)..... 109

Figure 4.8 Circular dichroism spectrum of PTX, DMβCD and PTXD-β-CD ICs.... 110

Figure 5.1 Formulation approaches for paclitaxel 122

Figure 5.2 Thermal changes during Lyophilization of Liposomes 137

Figure 5.3 Piepel's plot (A=HSPC, B=EggPC and C=Chol)..... 143

Figure 5.4 Two-component mixture plots:- A: effect of HSPC and EPC, B: effect of HSPC and Chol, C: effect of EPC and Chol 145

Figure 5.5 Major lipid compositions of HSPC (A) and EggPC (B), DSPC-Distearoyl-sn-glycerophosphocholine, DPPC – Dipalmitoyl-sn-glycerophosphocholine and POPC, Palmitoyl oleoyl-sn-glycerophosphocholine..... 146

Figure 5.6 Contour plot of effects of different components on particle size 146

Figure 5.7 Response surface plot of effects of different components on particle size.... 147

Figure 5.8 Piepel's plot (A=HSPC, B=EggPC and C=Chol)..... 150

Figure 5.9 Two-component mixture plots; A: effect of HSPC and EPC, B: effect of HSPC and Chol, C: effect of EPC and Chol 151

Figure 5.10 Contour plot of effects of different components on PDI 152

Figure 5.11 Response surface plot of effects of different components on PDI 153

LIST OF FIGURES

Figure 5.12 Surface plots showing optimum particle size and PDI at best trade-off for the constraints 155

Figure 5.13 Desirability Plot for Selection of Optimized Batch 155

Figure 5.14 Preparation of DLPLs 160

Figure 5.15 Particle size analysis for PLs 161

Figure 5.16 Zeta potential for PLs 162

Figure 5.17 Particle size analysis for DLPLs 162

Figure 5.18 Zeta potential for DLPLs 163

Figure 5.19 Cryo-TEM image of PLs (left) and DLPLs (right). 163

Figure 5.20 In vitro release of PTX from Taxol®, PLs and DLPLs up to 24 hr at 37 °C in phosphate buffer saline at pH 7.4 165

Figure 5.21 DSC thermogram of Paclitaxel 166

Figure 5.22 DSC thermogram of physical mixture of drug with lipids 166

Figure 5.23 DSC thermogram of DLPLs 167

Figure 5.24 FTIR spectra of Paclitaxel 168

Figure 5.25 FTIR of physical mixture of drug with lipids 168

Figure 5.26 FTIR of PEGylated liposomes (DLPLs) 169

Figure 5.27 Optical microscopy image of DLPLs before and after extrusion (above) and Drug crystal (below) at 20X 169

Figure 5.28 Stability study results for PLs and DLPLs 181

Figure 6.1 Graphical representation of Thio-ether linkage and conjugation of antibody to functionalized liposome 191

Figure 6.2 Schematic diagram of antibody digestion by Pepsin. 192

Figure 6.3 Antibody digestion and confirmation. 192

Figure 6.4 SDS-PAGE gel electrophoresis assembly. 195

Figure 6.5 DSPE-mPEG2000-Maleimide structure 208

Figure 6.6 FTIR of DSPE mPEG2000 maleimide 208

Figure 6.7 SDS PAGE gel after silver staining for antibody 212

Figure 6.8 Zeta potential for ILs 213

LIST OF FIGURES

Figure 6.9 Size distribution for ILs 214

Figure 6.10 In vitro release of PTX from DLPLs and ILs up to 24 hr at 37 °C in phosphate buffer saline at pH 7.4 215

Figure 7.1 Haemocytometer diagram indicating the 16 corner squares which should be used for counting. 225

Figure 7.2 Formation of formazan crystals 230

Figure 7.3 Specific binding of FITC labelled goat anti-mouse secondary antibody to the primary mAb (anti-FSHR mAb) indicating expression of FSHR in Caov3 cells, OVCAR3 cells and in SKOV3 cells 238

Figure 7.4 FACS analysis of FITC labelled goat anti-mouse secondary antibody to the primary mAb (anti-FSHR mAb) indicating expression of FSHR in (A) SKOV3 cells (B) OVCAR3 cells and (C) Caov3 cells. 239

Figure 7.5 Comparison of % relative mean fluorescence intensities of SKOV3 cells, OVCAR3 cells and Caov3 cells. (* indicates significantly high expression of FSHR in OVCAR3 and Caov3 cells $P<0.01$, as compared to SKOV3 cells). 239

Figure 7.6 Immunoreactivity of Fab′ fragments in Caov3 cell line: FACS analysis of cells treated at different concentrations of Fab′ (left) and its 2D overlay (right)..... 241

Figure 7.7 Immunoreactivity of immunoliposomes in Caov3 cell line: FACS analysis of cells treated at different concentrations of ILs (left) and its 2D overlay (right)... 242

Figure 7.8 Graphs representing % RFMI of Caov3 cells treated with different concentration of (A) Fab′ fragments and (B) radar representation of FACS values (C) ILs and (D) radar representation of FACS values..... 243

Figure 7.9 % cell viability at various concentrations of Taxol®, ICs, PLs, DLPLs, ILs and blank liposomes at 24 hr in SKOV3 cell line. 246

Figure 7.10 % cell viability at various concentrations of Taxol®, ICs, PLs, DLPLs, ILs and blank liposomes at 48 hr in SKOV3 cell line..... 246

Figure 7.11 % cell viability at various concentrations of Taxol®, ICs, PLs, DLPLs, ILs and blank liposomes at 24 hr in Caov3 cell line. 247

Figure 7.12 % cell viability at various concentrations of Taxol®, ICs, PLs, DLPLs, ILs and blank liposomes at 48 hr in Caov3 cell line 247

Figure 7.13 Comparative IC50 values for SKOV3 and Caov3 cell line after treatment with DLPLs and ILs at the end of 24 hr and 48 hr..... 248

LIST OF FIGURES

Figure 7.14 Cellular uptake studies of coumarin loaded non-targeted PLs and ILs carried out in FSHR expressing cell line (Caov3) and FSHR non-expressing cell line (SKOV3) 249

Figure 7.15 Specific uptake of coumarin 6–loaded non-targeted PLs and immunoliposomes (ILs) in FSHR-expressing Caov3 cells incubated with 10 µg/mL of PLs and ILs for 15, 30, 45, and 60 min. 250

Figure 7.16 FACS study for coumarin loaded non-targeted PLs and ILs carried out in FSHR non-expressing cell line (SKOV3) and FSHR expressing cell line (Caov3). 251

Figure 7.17 Specific uptake of coumarin 6–loaded non-targeted PLs and immunoliposomes (ILs) in FSHR-expressing Caov-3 cells incubated with 10 µg/mL of PLs and ILs for 15, 30, 45, and 60 min. 251

Figure 7.18 (A) Wound healing images in presence of TXT and various concentration of DLPLs. (B) Percent wound covered after 48 hr in comparison to untreated wound at 0 hr for ILs at various concentrations. ** $P < 0.01$ vs untreated as control. (C) Percent wound covere 252

Figure 7.19 FACS for untreated Caov3 cells 254

Figure 7.20 FACS for Caov3 cells treated with PI 254

Figure 7.21 FACS for Caov3 cells treated with 2nM Taxol® 255

Figure 7.22 FACS for Caov3 cells treated with 2nM DLPLs 255

Figure 7.23 FACS Caov3 for cells treated with 2nM ILs 256

Figure 7.24 (A) % SKOV3 and (B) Caov3 cell death after treatment with 2 nM Taxol, DLPLs and ILs for a period of 24 hr. (representative data) 256

Figure 7.25 % cell death after 24 and 48 hr treatment with different formulations in SKOV3 and Caov3 cells 257

Figure 7.26 Cell cycle analysis of control sample cells at 24 hr 258

Figure 7.27 Cell cycle analysis of Caov3 cells for Taxol® at 24 hr 259

Figure 7.28 Cell cycle analysis for Caov3 cells for DLPLs at 24 hr 259

Figure 7.29 Cell cycle analysis for Caov3 cells for ILs at 24 hr 260

Figure 7.30 Cell cycle analysis for Caov3 cells for control cells at 48 hr 260

Figure 7.31 Cell cycle analysis for Caov3 cells for Taxol® at 48 hr 261

Figure 7.32 Cell cycle analysis for Caov3 cells for DLPLs at 48 hr 261

Figure 7.33 Cell cycle analysis for Caov3 cells for ILs at 48 hr 262

Figure 7.34 Cell cycle analysis of Taxol®(PS), PLs and ILs in Skov3 cell line after 24 hr. 263

LIST OF FIGURES

Figure 7.35 Cell cycle analysis of Taxol®(PS), PLs and ILs in Skov3 cell line after 48 hr. 264

Figure 7.36 Cell cycle analysis of Taxol®(PS), PLs and ILs in Caov3 cell line after 24 hr. 264

Figure 7.37 Cell cycle analysis of Taxol®(PS), PLs and ILs in Caov3 cell line after 48 hr. 265

Figure 7.38% Cell in various phase of cell cycle after 24 hr treatment of skov3 cells with Taxol®(PS), DLPLs and ILs..... 265

Figure 7.39 % Cell in various phase of cell cycle after 48 hr treatment of skov3 cells with Taxol®(PS), DLPLs and ILs..... 266

Figure 7.40 % Cell in various phase of cell cycle after 24 hr treatment of caov3 cells with Taxol®(PS), DLPLs and ILs..... 266

Figure 7.41 % Cell in various phase of cell cycle after 48 hr treatment of Caov3 cells with Taxol®(PS), DLPLs and ILs 267

Figure 8.1: Hemolytic potential of DLPLs and ILs in comparison to Taxol® (*p<0.05)..... 276

Figure 8.2: Pharmacokinetic profile of PTX after single intravenous injection of Taxol®, DLPLs and ILs in rats. (A) full profile (B) magnified profile in concentration range of 1500-1000 ng/ml. Data are presented as mean ± SD..... 278