
CONTENTS

1. Introduction
 - 1.1 Introduction
 - 1.2 Aims to be achieved from the present study
 - 1.3 Hypothesis
 - 1.4 Objectives
 - 1.5 Plan of work
 - 1.6 References
2. Literature review
 - 2.1 Cancer
 - 2.1.1 Cancer statistics
 - 2.2 Tumor physiology
 - 2.2.1 Tumor growth
 - 2.2.2 Tumor vasculature and lymphatic system
 - 2.2.3 Barriers to drug delivery in tumors
 - 2.2.3.1 Reticuloendothelial system and mononuclear phagocytic system
 - 2.2.3.2 First pass renal filtering
 - 2.2.3.3 Heterogeneous blood flow
 - 2.2.3.4 High tumor interstitial pressure
 - 2.2.3.5 Extracellular matrix
 - 2.2.3.6 Intracellular transport
 - 2.3 Breast Cancer
 - 2.3.1 Antiestrogens
 - 2.3.2 EGFR and HER2/ Neu and Antiestrogen resistance
 - 2.3.3 Aromatase inhibitors
 - 2.3.4 Mechanisms of resistance
 - 2.3.5 Role of progesterone receptor and HER2/Neu
 - 2.4 Treatment of breast cancer
 - 2.4.1 Treatment options by stage
 - 2.4.2 Surgery
 - 2.4.3 Radiation therapy
 - 2.4.4 Chemotherapy

2.5 Targeted therapy

2.5.1 Nanoparticulate drug delivery system

2.5.1.1 Polymeric nanoparticles (Nanospheres and nanocapsules)

2.5.1.2 Liposomes

2.5.1.3 Micelles

2.5.1.4 Dendrimers

2.6 Targeting

2.6.1 Passive targeting

2.6.1.1 Size

2.6.1.2 Particle shape

2.6.1.3 Surface characteristics

2.6.1.4 PEGylation

2.6.1.5 Limitations of passive targeting

2.6.2 Active targeting

2.6.2.1 Folate

2.6.2.2 Transferrin

2.6.2.3 Aptamers

2.6.2.4 Antibodies (monoclonal antibodies)

2.6.2.5 Peptides

2.6.2.6 Limitations of active targeting

2.7 Poly lactic-co-glycolic acid (PLGA)

2.8 Polycaprolactone

2.9 Anastrozole

2.10 Exemestane

2.11 References

3. Analytical methods

3.1 Materials

3.2 Estimation method for anastrozole

3.2.1 High performance liquid chromatography

3.2.2 Preparation of standard stock solutions

3.2.3 Preparation of calibration curve

3.2.4 Analytical method validation

3.2.4.1	Linearity
3.2.4.2	Accuracy
3.2.4.3	Precision
3.2.5	Estimation of anastrozole in nanoparticulate formulations
3.3	Estimation method for exemestane
3.3.1	High performance liquid chromatography
3.3.2	Preparation of standard stock solutions
3.3.3	Preparation of calibration curve
3.3.4	Analytical method validation
3.2.4.1	Linearity
3.2.4.2	Accuracy
3.2.4.3	Precision
3.3.5	Estimation of exemestane in nanoparticulate formulations
3.4	References
4.	Formulation, optimization and characterization of nanoparticulate formulation
4.1	Introduction
4.2	Materials
4.3	Synthesis of polymer and conjugates
4.3.1	Synthesis of PLGA-PEG conjugate
4.3.2	Synthesis of cPCL
4.3.3	Synthesis of PCL-PEG conjugate
4.4	Characterization of polymer and conjugates
4.4.1	FTIR spectroscopy
4.4.2	NMR spectroscopy
4.4.3	Molecular weight determination
4.5	Formulation and optimization of ATZ loaded PLGA NPs
4.5.1	Preparation of ATZ loaded PLGA NPs
4.5.2	Drug content and percentage drug entrapment
4.5.3	Particle size
4.5.4	Preliminary optimization of ATZ loaded PLGA NPs
4.5.4.1	Selection of organic solvent
4.5.4.2	Selection of volume of organic solvent

- 4.5.4.3 Selection of surfactant
- 4.5.5 Optimization
 - 4.5.5.1 Experimental design for optimization of key formulation variables
 - 4.5.5.2 Contour plots
 - 4.5.5.3 Response surface plots
 - 4.5.5.4 Check point analysis
 - 4.5.5.5 Desirability criteria
 - 4.5.5.6 Normalized error determination
- 4.5.6 Lyophilization and optimization of cryoprotectant
- 4.6 Formulation and optimization of ATZ loaded cPCL NPs
 - 4.6.1 Preparation of ATZ loaded cPCL NPs
 - 4.6.2 Drug content and percentage drug entrapment
 - 4.6.3 Particle size
 - 4.6.4 Preliminary optimization of ATZ loaded cPCL NPs
 - 4.6.4.1 Selection of organic solvent
 - 4.6.4.2 Selection of volume of organic solvent
 - 4.6.4.3 Selection of surfactant
 - 4.6.5 Optimization
 - 4.6.5.1 Experimental design for optimization of key formulation variables
 - 4.6.5.2 Contour plots
 - 4.6.5.3 Response surface plots
 - 4.6.5.4 Check point analysis
 - 4.6.5.5 Desirability criteria
 - 4.6.5.6 Normalized error determination
 - 4.6.6 Lyophilization and optimization of cryoprotectant
- 4.7 Formulation and optimization of EXE loaded PLGA NPs
 - 4.7.1 Preparation of EXE loaded PLGA NPs
 - 4.7.2 Drug content and percentage drug entrapment
 - 4.7.3 Particle size
 - 4.7.4 Preliminary optimization of EXE loaded PLGA NPs
 - 4.7.4.1 Type of organic solvent
 - 4.7.4.2 Selection of surfactant
 - 4.7.4.3 Concentration of surfactant

4.7.5 Optimization	
4.7.5.1 Experimental design for optimization of key formulation variables	
4.7.5.2 Contour plots	
4.7.5.3 Response surface plots	
4.7.5.4 Check point analysis	
4.7.5.5 Desirability criteria	
4.7.5.6 Normalized error determination	
4.7.6 Lyophilization and optimization of cryoprotectant	
4.8 Formulation and optimization of EXE loaded cPCL NPs	
4.8.1 Preparation of EXE loaded cPCL NPs	
4.8.2 Drug content and percentage drug entrapment	
4.8.3 Particle size	
4.8.4 Preliminary optimization of EXE loaded cPCL NPs	
4.8.4.1 Type of organic solvent	
4.8.4.2 Selection of surfactant	
4.8.4.3 Concentration of surfactant	
4.8.5 Optimization	
4.8.5.1 Experimental design for optimization of key formulation variables	
4.8.5.2 Contour plots	
4.8.5.3 Response surface plots	
4.8.5.4 Check point analysis	
4.8.5.5 Desirability criteria	
4.8.5.6 Normalized error determination	
4.8.6 Lyophilization and optimization of cryoprotectant	
4.9 Characterization of optimized nanoparticulate formulation	
4.9.1 Zeta potential	
4.9.2 Transmission electron microscope studies	
4.9.3 Differential scanning calorimetric (DSC) studies	
4.9.4 In vitro drug release studies	
4.9.5 Stability studies	
4.10 Results and discussion	
4.10.1 Characterization of PLGA-PEG conjugate	
4.10.2 Characterization of cPCL	

- 4.10.3 Characterization of PCL-PEG conjugate
- 4.11 Formulation and optimization of ATZ loaded PLGA NPs
 - 4.11.1 Preliminary optimization of ATZ loaded PLGA NPs
 - 4.11.1.1 Selection of organic solvent
 - 4.11.1.2 Selection of volume of organic solvent
 - 4.11.1.3 Selection of surfactant
 - 4.11.2 Optimization of ATZ loaded PLGA NPs using 3^3 factorial design
 - 4.11.2.1 Contour plots
 - 4.11.2.2 Response surface plots
 - 4.11.2.3 Desirability criteria
 - 4.11.2.4 Checkpoint analysis and normalized error
 - 4.11.3 Lyophilization and optimization of cryoprotectants
- 4.12 Formulation and optimization of ATZ loaded cPCL NPs
 - 4.12.1 Preliminary optimization of ATZ loaded cPCL NPs
 - 4.12.1.1 Selection of organic solvent
 - 4.12.1.2 Selection of volume of organic solvent
 - 4.12.1.3 Selection of surfactant
 - 4.12.2 Optimization of ATZ loaded cPCL NPs using 3^3 factorial design
 - 4.12.2.1 Contour plots
 - 4.12.2.2 Response surface plots
 - 4.12.2.3 Desirability criteria
 - 4.12.2.4 Checkpoint analysis and normalized error
 - 4.12.3 Lyophilization and optimization of cryoprotectants
- 4.13 Formulation and optimization of EXE loaded PLGA NPs
 - 4.13.1 Preliminary optimization of EXE loaded PLGA NPs
 - 4.13.1.1 Selection of organic solvent
 - 4.13.1.2 Selection of surfactant
 - 4.13.1.3 Selection of surfactant concentration
 - 4.13.2 Optimization of EXE loaded PLGA NPs using BBD
 - 4.13.2.1 Contour plots
 - 4.13.2.2 Response surface plots
 - 4.13.2.3 Desirability criteria
 - 4.13.2.4 Checkpoint analysis and normalized error

- 4.13.3 Lyophilization and optimization of cryoprotectants
- 4.14 Formulation and optimization of EXE loaded cPCL NPs
 - 4.14.1 Preliminary optimization of EXE loaded cPCL NPs
 - 4.14.1.1 Selection of organic solvent
 - 4.14.1.2 Selection of surfactant
 - 4.14.1.3 Selection of surfactant concentration
 - 4.14.2 Optimization of EXE loaded cPCL NPs using BBD
 - 4.14.2.1 Contour plots
 - 4.14.2.2 Response surface plots
 - 4.14.2.3 Desirability criteria
 - 4.14.2.4 Checkpoint analysis and normalized error
 - 4.14.3 Lyophilization and optimization of cryoprotectants
- 4.15 Characterization of ATZ loaded PLGA NPs
 - 4.15.1 Zeta potential
 - 4.15.2 Transmission electron microscopy (TEM)
 - 4.15.3 Differential scanning calorimetry (DSC)
 - 4.15.4 In vitro drug release studies
 - 4.15.5 Stability studies
- 4.16 Characterization of ATZ loaded cPCL NPs
 - 4.16.1 Zeta potential
 - 4.16.2 Transmission electron microscopy (TEM)
 - 4.16.3 Differential scanning calorimetry (DSC)
 - 4.16.4 In vitro drug release studies
 - 4.16.5 Stability studies
- 4.17 Characterization of EXE loaded PLGA NPs
 - 4.17.1 Zeta potential
 - 4.17.2 Transmission electron microscopy (TEM)
 - 4.17.3 Differential scanning calorimetry (DSC)
 - 4.17.4 In vitro drug release studies
 - 4.17.5 Stability studies
- 4.18 Characterization of EXE loaded cPCL NPs
 - 4.18.1 Zeta potential
 - 4.18.2 Transmission electron microscopy (TEM)

4.18.3	Differential scanning calorimetry (DSC)
4.18.4	In vitro drug release studies
4.18.5	Stability studies
4.19	References
5.	Pegylation and surface functionalization
5.1	Materials
5.2	Cell lines
5.3	Methods
5.3.1	Pegylation of nanoparticles
5.3.2	Phagocytic uptake
5.3.3	Surface functionalization of NPs with ER antibody
5.4	Results and discussion
5.4.1	Physicochemical characterization of ATZ loaded PLGA NPs
5.4.2	Optimization of ATZ loaded PLGA NPs containing different PEG ratio based on PDE, PS and phagocytic uptake studies
5.4.3	Physicochemical characterization of ImmunoNPs
5.4.4	Physicochemical characterization of EXE loaded PCL NPs
5.4.5	Optimization of EXE loaded PCL NPs containing different PEG ratio based on PDE, PS and phagocytic uptake studies
5.4.6	Physicochemical characterization of ImmunoNPs
5.5	References
6.	Cell culture studies
6.1	Introduction
6.1.1	Cellular uptake
6.1.2	Cytotoxicity studies
6.1.3	Apoptosis
6.1.4	Cell cycle analysis
6.2	Materials
6.3	Cell lines
6.4	Methods
6.4.1	Receptor expression analysis by Western Blot
6.4.2	Qualitative cellular uptake by fluorescent microscopy

6.4.3	Quantitative cellular uptake by flow cytometry
6.4.4	In vitro cytotoxicity studies by MTT Assay
6.4.5	In vitro apoptosis study
6.4.6	Cell cycle analysis by flow cytometry
6.5	Results and discussion
6.5.1	Receptor expression in cell lines
6.5.2	Qualitative and quantitative cellular uptake
6.5.3	In vitro cytotoxicity studies by MTT Assay
6.5.4	Apoptosis studies
6.5.5	Cell cycle analysis
6.6	References
7.	In vivo biodistribution studies
7.1	Introduction
7.2	Materials
7.3	Methods
7.3.1	Radioiodination of antibody
7.3.2	Determination of labeling efficiency by electrophoresis
7.3.3	Purification of radiolabeled antibody
7.3.4	Conjugation of iodinated antibody and TME on NPs
7.3.5	Stability of radiolabelled conjugates
7.3.6	Biodistribution studies
7.3.7	Statistical analysis
7.4	Results and discussion
7.4.1	Stability of radioiodinated complexes
7.4.2	Biodistribution studies
7.4.2.1	Biodistribution of ^{125}I -Tyr PLGA NPs and ^{125}I -ER Ab PLGA NPs
7.4.2.2	Biodistribution of ^{125}I -Tyr PCL NPs and ^{125}I -ER Ab PCL NPs
7.5	References
8.	Summary and Conclusion
8.1	Summary
8.2	Conclusion