# 1. Introduction

1.1 Introduction

1.2 Aims and Objectives

References

# 2. Review of Literature

2.1 Cancer and Nanotechnology

2.2 Block Copolymeric Micelles in Drug Delivery

2.2.1 Introduction

2.2.2 Types of amphiphilic block copolymers

2.2.3 Formation of block copolymeric micelles

2.2.4 Stability of micelles

2.2.5 Functionalized block copolymeric micelles

2.2.5.1 Ligand conjugated micelles

2.2.5.2 Stimuli-responsive polymeric micelles

2.3 Drug Profile

2.4 Peptide Profile

References

# 3. Analytical Method Development

3.1 Materials

3.2 Analytical Method Development of FTO

3.2.1 Analytical method development by UV Spectroscopy

3.2.1.1 Calibration curve of FTO in Acetonitrile (ACN) and Phosphate buffer saline (PBS) pH 7.4

3.2.1.2 Analytical method validation

3.2.1.3 Results and discussion

3.2.2 Analysis method development of FTO in cell lysate by HPLC method
3.2.2.1 HPLC conditions 43
3.2.2.2 Stock solution and working standard solutions 44
3.2.2.3 Sample preparation 44
3.2.2.4 Calibration curve 44
3.2.2.5 Precision and accuracy 45
3.2.2.6 Extraction efficiency 45
3.2.2.7 Results and discussion 45
3.3 Analytical Method Development of Poly(ethylene glycol) 48
  3.3.1 Calibration curve of polyethylene glycol 48
  3.3.2 Results and discussion 48
3.4 Analytical Method Development of YIGSR-NH$_2$ & EILDV-NH$_2$ 50
  3.4.1 Calibration curve of peptides (YIGSR-NH$_2$ & EILDV-NH$_2$) 50
  3.4.2 Results and discussion 51
References 53

4. Synthesis & Characterization of PEG-PCL Di-block Copolymer 55
4.1 Materials 55
4.2 Synthesis of PEG-PCL Di-block Copolymer 55
4.3 Characterization of PEG-PCL Di-block Copolymer 57
  4.3.1 Nuclear magnetic resonance ($^1$H-NMR) 57
  4.3.2 Gel permeation chromatography (GPC) 57
  4.3.3 Fourier transform infrared spectroscopy (FTIR) 58
4.4 Results and Discussion 58
References 71

5. Formulation Design & Evaluation of Methoxy PEG-PCL Micelles 73
5.1 Materials 73
5.2 Preparation of ETO Loaded MPEG-PCL (MPCL) Micelles 73
5.3 Evaluation of MPCL Micelles 74
  5.3.1 Particle size and zeta potential 74
  5.3.2 Determination of percent entrapment efficiency and percent drug loading 74
  5.3.3 Critical micelle concentration 74

II
5.3.4 Fixed aqueous layer thickness
5.3.5 In vitro stability study
5.3.6 Hemolysis study
5.3.7 PEG surface density
5.4 Result and discussion
5.4.1 Preparation of MPCL micelles
5.4.2 Evaluation of MPCL micelles
5.4.2.1 Particle size and zeta potential
5.4.2.2 Critical micelle concentration
5.4.2.3 Fixed aqueous layer thickness
5.4.2.4 In vitro stability studies
5.4.2.5 PEG surface density
5.4.2.6 Hemolysis study
5.5 Selection of MPCL micelles

References

6. Assembly & Characterization of Peptide Conjugated PEG-PCL micelles
6.1 Materials
6.2 Assembly of peptide conjugated micelles
6.3 Characterization of micellar formulation
6.3.1 Differential scanning calorimetry
6.3.2 X-ray diffractogram
6.3.3 Transmission electron microscopy
6.3.4 Lyophilization
6.3.5 In-vitro release studies
6.3.6 Stability studies
6.4 Results and Discussion
6.4.1 Assembly of peptide conjugated micelles
6.4.2 Characterization of micelles
6.4.2.1 Differential scanning calorimetry
6.4.2.2 X-ray Diffraction
6.4.2.3 Transmission electron microscopy
6.4.2.4 Lyophilization study
6.4.2.5 In vitro release studies
6.4.2.6 Stability studies
References

7. In Vitro Cell Line Studies
7.1 Cell and culture conditions
7.2 Materials
7.3 Methods
  7.3.1 Cytotoxicity assay
  7.3.2 Cytopathic study
  7.3.3 Colony forming assay
  7.3.4 Cell migration assay
  7.3.5 Cell adhesion study
  7.3.6 Confocal microscopy
  7.3.7 Cell uptake study
  7.3.8 Cell cycle analysis by flow cytometry
7.4 Results and discussion
  7.4.1 Cytotoxicity assay
  7.4.2 Cytopathic study
  7.4.3 Colony forming assay
  7.4.4 Cell migration assay
  7.4.5 Cell adhesion study
  7.4.6 Confocal microscopy
  7.4.7 Cell uptake studies
  7.4.8 Cell cycle analysis by flow cytometry
References

8. In Vivo Studies
8.1 Biodistribution study
  8.1.1 Materials
  8.1.2 Animals
  8.1.3 Radiolabeling of ETO and micellar formulations
  8.1.4 Determination of labeling efficiency
References
8.1.5 In vitro stability of labeled complexes
8.1.6 Tumor implantation
8.1.7 Biodistribution study
8.2 Experimental metastasis study
8.2.1 Animals
8.2.2 In vitro treatment of B16F10 melanoma cells with formulations and its effect on inhibition of lung metastasis
8.2.3 In vivo treatment of B16F10 melanoma and its effect on inhibition of lung metastasis with formulations
8.2.4 Histopathology study
8.3 Results and discussions
8.3.1 Biodistribution study
8.3.1.1 Radiolabeling efficiency of ETO and micellar formulations
8.3.1.2 In vitro stability of labeled complexes
8.3.1.3 Biodistribution study
8.3.2 Experimental metastasis
8.3.2.1 In vitro treatment of B16F10 melanoma cells with formulations and its effect on inhibition of lung metastasis
8.3.2.2 In vivo treatment of B16F10 melanoma and its effect on inhibition of lung metastasis with formulations
8.3.2.3 Histopathology studies
References

9. Summary & Conclusion

Presentations & Publications XX