



Table of Contents

List of Tables	
List of Figures	XI
Abbreviations	XVIII
1. Introduction	
1.1 Introduction	1
1.2 Aims and Objectives	4
References	5
2. Review of Literature	
2.1 Cancer and Nanotechnology	7
2.2 Block Copolymeric Micelles in Drug Delivery	9
2.2.1 Introduction	9
2.2.2 Types of amphiphilic block copolymers	9
2.2.3 Formation of block copolymeric micelles	10
2.2.4 Stability of micelles	12
2.2.5 Functionalized block copolymeric micelles	14
2.2.5.1 Ligand conjugated micelles	15
2.2.5.2 Stimuli-responsive polymeric micelles	20
2.3 Drug Profile	23
2.4 Peptide Profile	27
References	28
3. Analytical Method Development	
3.1 Materials	38
3.2 Analytical Method Development of ETO	38
3.2.1 Analytical method development by UV Spectroscopy	38
3.2.1.1 Calibration curve of ETO in Acetonitrile (ACN) and Phosphate buffer saline (PBS) pH 7.4	38
3.2.1.2 Analytical method validation	38
3.2.1.3 Results and discussion	39
3.2.2 Analysis method development of ETO in cell lysate by HPLC method	43

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-NH2 & EILDV-NH2)	50
3.4.2	Results and discussion	51
	References	53
4. Synthesis & Characterization of PEG-PCL Di-block Copolymer		
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		
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

5.3.4 Fixed aqueous layer thickness	75
5.3.5 In vitro stability study	75
5.3.6 Hemolysis study	76
5.3.7 PEG surface density	77
5.4 Result and discussion	78
5.4.1 Preparation of MPCL micelles	78
5.4.2 Evaluation of MPCL micelles	92
5.4.2.1 Particle size and zeta potential	92
5.4.2.2 Critical micelle concentration	94
5.4.2.3 Fixed aqueous layer thickness	97
5.4.2.4 In vitro stability studies	101
5.4.2.5 PEG surface density	105
5.4.2.6 Hemolysis study	107
5.5 Selection of MPCL micelles	110
References	111

6. Assembly & Characterization of Peptide Conjugated PEG-PCL micelles

6.1 Materials	117
6.2 Assembly of peptide conjugated micelles	117
6.3 Characterization of micellar formulation	118
6.3.1 Differential scanning calorimetry	118
6.3.2 X-ray diffractogram	118
6.3.3 Transmission electron microscopy	118
6.3.4 Lyophilization	119
6.3.5 In-vitro release studies	119
6.3.6 Stability studies	120
6.4 Results and Discussion	120
6.4.1 Assembly of peptide conjugated micelles	120
6.4.2 Characterization of micelles	124
6.4.2.1 Differential scanning calorimetry	124
6.4.2.2 X-ray Diffraction	125
6.4.2.3 Transmission electron microscopy	127
6.4.2.4 Lyophilization study	128

6.4.2.5 <i>In vitro</i> release studies	133
6.4.2.6 Stability studies	136
References	140
7. In Vitro Cell Line Studies	
7.1 Cell and culture conditions	143
7.2 Materials	143
7.3 Methods	143
7.3.1 Cytotoxicity assay	143
7.3.2 Cytopathic study	144
7.3.3 Colony forming assay	144
7.3.4 Cell migration assay	145
7.3.5 Cell adhesion study	145
7.3.6 Confocal microscopy	146
7.3.7 Cell uptake study	146
7.3.8 Cell cycle analysis by flow cytometry	147
7.4 Results and discussion	148
7.4.1 Cytotoxicity assay	148
7.4.2 Cytopathic study	160
7.4.3 Colony forming assay	168
7.4.4 Cell migration assay	171
7.4.5 Cell adhesion study	175
7.4.6 Confocal microscopy	178
7.4.7 Cell uptake studies	181
7.4.8 Cell cycle analysis by flow cytometry	184
References	188
8. In Vivo Studies	
8.1 Biodistribution study	192
8.1.1 Materials	192
8.1.2 Animals	192
8.1.3 Radiolabeling of ETO and micellar formulations	192
8.1.4 Determination of labeling efficiency	193

8.1.5 <i>In vitro</i> stability of labeled complexes	193
8.1.6 Tumor implantation	194
8.1.7 Biodistribution study	194
8.2 Experimental metastasis study	194
8.2.1 Animals	194
8.2.2 <i>In vitro</i> treatment of B16F1O melanoma cells with formulations and its effect on inhibition of lung metastasis	195
8.2.3 <i>In vivo</i> treatment of B16F1O melanoma and its effect on inhibition of lung metastasis with formulations	195
8.2.4 Histopathology study	196
8.3 Results and discussions	196
8.3.1 Biodistribution study	196
8.3.1.1 Radiolabeling efficiency of ETO and micellar formulations	196
8.3.1.2 <i>In vitro</i> stability of labeled complexes	197
8.3.1.3 Biodistribution study	199
8.3.2 Experimental metastasis	211
8.3.2.1 <i>In vitro</i> treatment of B16F1O melanoma cells with formulations and its effect on inhibition of lung metastasis	211
8.3.2.2 <i>In vivo</i> treatment of B16F1O melanoma and its effect on inhibition of lung metastasis with formulations	214
8.3.2.3 Histopathology studies	218
References	220
9. Summary & Conclusion	223

Presentations & Publications

XX