



INDEX

TABLE OF CONTENTS-----	i
LIST OF FIGURES-----	vii
LIST OF TABLES-----	xiii
LIST OF ABBREVIATIONS-----	xv
CHAPTER I. Introduction-----	1-40
1.1. Overview-----	1
1.2. Research Envisaged-----	6
1.3. Thesis structure-----	7
1.4. Literature Review-----	8
1.4.1. <i>Block copolymers and their intrinsic properties</i> -----	8
1.4.2. <i>General considerations for micelles and vesicles</i> <i>Formation</i> -----	10
1.4.2.a. Theoretical aspects-----	10
1.4.2.b. Experimental consideration-----	11
1.4.3. <i>Specific characteristics of micelles and vesicles</i> -----	11
1.4.3.a. Spherical micelles-----	12
1.4.3.b. Block copolymer vesicles or polymersomes-----	13
1.4.3.c. Other morphologies and recent developments with block Copolymer-----	14
1.4.4. <i>Characterization techniques</i> -----	16
1.4.5. <i>Therapeutic application of self-assembled micelles</i> <i>and vesicles or polymersomes</i> -----	16
1.4.5.1. Block copolymer micelles-----	17
1.4.5.1.a. Drug solubility enhancement-----	17
1.4.5.1.a.1. <i>Solubility enhancement</i> <i>via physical interactions</i> -----	17
1.4.5.1.a.2. <i>Solubility enhancement</i> <i>via covalent bridging</i> -----	19
1.4.5.1.b. Passive drug targeting-----	20
1.4.5.1.b.1. <i>Polypeptide-based block copolymer</i> <i>micelle</i> -----	20
1.4.5.1.b.2. <i>Polyester-based block copolymer</i> <i>micelle</i> -----	22

1.4.5.1.b.3. <i>Poloxamer-based block copolymer micelle</i> -----	23
1.4.5.1.c. Active drug targeting-----	24
1.4.5.1.c.1. <i>Folate functionalization</i> -----	24
1.4.5.1.c.2. <i>Peptide functionalization</i> -----	26
1.4.5.1.c.3. <i>Antibody functionalization</i> -----	27
1.4.5.1.c.4. <i>Carbohydrates functionalization</i> ---	27
1.4.5.2. Block copolymer vesicles or polymersomes-----	28
References-----	30-40
CHAPTER II. Synthesis of Polypeptide-block-Polysaccharide Copolymer -----	41-63
2.1. Introduction-----	41
2.2. Materials-----	44
2.3. Synthesis of α -alkyne-HYA (HYACCH) by reductive amination-----	45
2.4. Acid treatment of α -alkyne-HYA (HYACCH)-----	45
2.5. Synthesis of 1-azido-3-aminopropane-----	45
2.6. Synthesis of α -azido PBLG (PBLG-N ₃)-----	46
2.7. Synthesis of HYA- <i>b</i> -PBLG block Co-polymer by Huisgen's 1,3-Dipolar Cycloaddition ("Click" Reaction)-----	47
2.8. Characterization-----	47
2.8.1. <i>Fourier transform infrared spectroscopy</i> -----	47
2.8.2. <i>¹H-NMR</i> -----	47
2.8.3. <i>Size exclusion chromatography measurement</i> -----	48
2.8.4. <i>Differential scanning calorimetry measurement</i> -----	48
2.9. Results and Discussion-----	48
2.9.1. <i>The α-Alkyne-HYA by Reductive amination</i> -----	48
2.9.2. <i>The α-Azido PBLG (PBLG-N₃)</i> -----	52
2.9.3. <i>Click Reaction</i> -----	56
2.10. Conclusion-----	58
References-----	59-63

CHAPTER III. Self-assembly Behavior of Block Copolymer in Aqueous Solution----- 64-87

3.1. Introduction-----	64
3.2. Experimental-----	66
3.2.1. <i>Polymersomes formation</i> -----	66
3.2.2. <i>Process optimization</i> -----	66
• <i>Effect of PBLG₂₃-b-HYA₁₀ copolymer concentration in DMSO solvent</i> -----	66
• <i>Effect of final concentration of PBLG₂₃-b-HYA₁₀ copolymer in nanoprecipitation</i> -----	67
3.2.3. <i>Effect of dilution on preformed PBLG₂₃-b-HYA₁₀ vesicle</i> -----	67
3.2.4. <i>Stability study</i> -----	67
3.2.5. <i>Lyophilization</i> -----	67
3.3. Characterization-----	68
3.3.1. <i>Dynamic and static light scattering</i> -----	68
3.3.2. <i>Small angle neutron scattering</i> -----	69
3.3.3. <i>Freeze fracture TEM</i> -----	70
3.3.4. <i>Atomic force microscopy</i> -----	70
3.4. Results and Discussion-----	70
3.5. Conclusion-----	82
References-----	83-87

CHAPTER IV. Development and Characterization of Doxorubicin Loaded Polymersomes (PolyDOX) and Preliminary Cell line Studies on C6 glioma cells----- 88-113

4.1. Introduction	88
4.2. Materials	89
4.3. Formulation DOX loaded polymersomes (PolyDOX)	90
4.4. Characterization	90
4.4.1. <i>Dynamic light scattering</i>	90
4.4.2. <i>Microscopy</i>	91
4.4.3. <i>pH effect on zeta potential and particle size of PolyDOX</i>	91

4.4.4. <i>In vitro</i> release studies	92
4.5. Stability studies	92
4.6. Lyophilization process	92
4.7. Cell line studies on C6 rat glioma cells	93
4.7.1. Cell culture	93
4.7.2. Effect on cell morphology after treatment	93
4.7.3. Cell viability	93
4.7.4. Cell uptake studies (Fluorescence Microscopy)	94
4.7.5. Cell uptake studies (Flow Cytometry)	94
4.8. Results and Discussion	94
4.8.1. Doxorubicin loaded polymersomes (PolyDOX)	94
4.8.2. <i>In vitro</i> cell line studies on C6 rat glioma cancer cell	104
4.9. Conclusion	109
References	110-113
CHAPTER V. <i>In Vitro</i> Cell Line Studies and Tumor Regression on DMBA Induced Breast Tumor Bearing Rats—	114-139
5.1. Introduction-----	114
5.2. Materials-----	115
5.3. Cell culture-----	115
5.4. CD44 expression (FACS analysis)-----	116
5.5. DOX uptake assay-----	116
5.5.1. Microscopy-----	116
5.5.2. Flow-cytometric Analysis-----	116
5.6. Chemosensitivity Assay-----	117
5.7. Competitive inhibition of PolyDOX in presence of free hyaluronan-----	117
5.8. Reactive oxygen Species (ROS) measurement-----	118
5.9. <i>In Vivo</i> Evaluation of Antitumor Efficacy of PolyDOX-----	118
5.10. Assessment of cardiotoxicity-----	119
5.11. Statistical analysis-----	119
5.12. Results and Discussion-----	119
5.12.1. Analysis of CD44 expression-----	119
5.12.2. Analysis of DOX uptake by fluorescence microscopy-----	120

5.12.3. Analysis of DOX uptake by flow cytometry-----	123
5.12.4. Cytotoxicity Study-----	126
5.12.5. DOX induced Reactive Oxygen Species (ROS) Generation-----	130
5.12.6. Anti-tumor activity of PolyDOX-----	131
5.13. Conclusion-----	134
References-----	135-139
CHAPTER VI. PolyDOX: <i>In Vivo</i> Animal Study on EAT	
Bearing Mice-----	140-176
6.1. Introduction-----	140
6.2. Materials-----	141
6.3. Radiolabeling of DOX, PolyDOX and Blank polymersomes (Blank-POLY)-----	142
6.4. Transchelation of complexes-----	143
6.5. <i>In vitro</i> and <i>in vivo</i> stability of radiolabeled compounds-----	143
6.6. Stability study of PolyDOX in serum-----	144
6.7. Blood clearance study of ^{99m} Tc-PolyDOX and ^{99m} Tc-DOX-----	144
6.8. Expression of CD44 level in EAT cells-----	144
6.9. Biodistribution on EAT tumor bearing mice-----	145
6.10. Side effects of PolyDOX-----	145
6.11. Hemolysis activity of PolyDOX-----	147
6.12. Antitumor activity of PolyDOX-----	147
6.13. Gamma Scintigraphy study of PolyDOX-----	148
6.14. Statistical analysis-----	148
6.15. Results and Discussion-----	149
6.15.1 ^{99m} Tc labelled PolyDOX, Blank-POLY and free DOX-----	149
6.15.2 Stability of PolyDOX and Blank-POLY in serum-----	151
6.15.3 Blood Clearance study of ^{99m} Tc labeled compounds-----	152
6.15.4 Ehrlich Ascites Tumor-----	154
6.15.5 Biodistribution study of ^{99m} Tc-labelled compounds-----	155
6.15.6 Toxicity of PolyDOX-----	159
6.15.7 Hemolysis effect of PolyDOX and Blank-POLY-----	164

6.15.8 Antitumor activity of PolyDOX and DOX-----	165
6.15.9 Gamma Scintigraphy study-----	168
6.16. Conclusion-----	169
References-----	169-176
Chapter VII. Docetaxel Loaded Polymersomes: Development and Characterization <i>In Vitro</i> and <i>In Vivo</i>-----	177-204
7.1. Introduction-----	177
7.2. Materials -----	178
7.3. Formulation of docetaxel loaded polymersomes (PolyDOCE) and docetaxel solution (DS)-----	178
7.4. <i>In vitro</i> release studies-----	179
7.5. Stability studies-----	179
7.6. Lyophilization process-----	180
7.7. <i>In vitro</i> cytotoxicity studies on MCF-7 and U87-----	180
7.8. ^{99m} Tc labeling of DOC and PolyDOC-----	181
7.9. Transchelation of complexes-----	182
7.10. <i>In vitro</i> and <i>in vivo</i> stability of radiolabel compounds-----	182
7.11. Pharmacokinetic study of ^{99m} Tc-PolyDOC and ^{99m} Tc-DS-----	183
7.12. Hemolysis effect-----	183
7.13. Biodistribution study of ^{99m} Tc-PolyDOC and ^{99m} Tc-DS-----	184
7.14. Statistical analysis-----	184
7.15. Results and Discussion-----	185
7.15.1 Docetaxel loaded polymersomes(PolyDOC)-----	185
7.15.2 Cytotoxicity study-----	191
7.15.3 ^{99m} Tc-Labeled DS and PolyDOC-----	193
7.15.4 Blood Clearance study of ^{99m} Tc labeled compounds-----	195
7.15.5 Hemolysis effect of PolyDOC and DS-----	197
7.15.6 Biodistribution study of ^{99m} Tc-labeled compounds-----	198
7.16. Conclusion-----	200
References-----	201-204
Chapter VIII. Summary and Conclusion-----	205-218
APPENDIX I (List of Presentations and Publications)-----	219-210