

CONTENTS

List of tables

List of figures

Abbreviations

Preface

Chapters	Page No.
1. Introduction.....	1-16
2. Literature review.....	17-65
2.1. Molecular targets available for antithrombotic nanomedicine.....	17-21
2.2. Nanoplatforms developed for anti-thrombotic therapy.....	21-22
2.2.1. Liposomes	22-24
2.2.2. Micelles	24-25
2.2.3. Polymeric nanoparticles	25
2.2.4. Material-based nanoparticles (inorganic nanoparticles)	25-26
2.2.5. Other biological vectors.....	26-27
2.3. Strategies involved in nanocarrier based thrombus targeting	27-29
2.3.1. Passive targeted antithrombotic nanotherapeutics	30
2.3.1.1. Shear activated nanotherapeutics.....	30-31
2.3.1.2. Stimulus-sensitive antithrombotic nanomedicines.....	31-33
2.3.2. Active targeted antithrombotic nanotherapeutics.....	34
2.3.2.1. Ligand-based thrombus targeted nanomedicines.....	34-40
2.3.3. Miscellaneous strategies involved in thrombus targeted nanomedicine.....	40
2.3.3.1. Sonothrombolysis	40-42
2.3.3.2. Magneto-thrombolysis.....	42-45
2.3.3.3. Dual targeted antithrombotic nanomedicines.....	45-46
2.4. Antithrombotic nanomedicine for theranostics applications	47-50
2.5. Mesoporous silica nanoparticle (MSN).....	50-51
2.5.1. Methods of preparation of mesoporous silica nanoparticle.....	52
2.5.1.1. Sol-gel method.....	52
2.5.1.2. Template assisted method.....	52
2.5.1.3. Chemical etching method.....	53
2.5.1.4. Microwave assisted method.....	53
2.5.2. Biosimilar's profile	54
2.5.2.1. Chemical structure.....	54
2.5.2.2. Description.....	54
2.5.2.3. Molecular weight.....	54
2.5.2.4. Molecular formula.....	54
2.5.2.5. Half-life.....	54

2.5.2.6.Mechanism of action.....	54
2.5.2.7.Indication.....	54
2.5.2.8.Side effects.....	55
2.5.3. Tetraethyl orthosilicate	55
2.5.3.1.Chemical structure.....	55
2.5.3.2.Description.....	55
2.5.3.3.IUPAC name.....	55
2.5.3.4.Molecular formula.....	55
2.5.3.5.Molecular weight.....	55
2.5.3.6.Physical properties.....	55
2.5.3.7.Metabolism.....	56
2.5.3.8.Uses.....	56
2.5.4. Cetyltrimethyl ammonium bromide	56
2.5.4.1.Chemical structure.....	56
2.5.4.2.Description.....	56
2.5.4.3.IUPAC name.....	56
2.5.4.4.Molecular formula.....	56
2.5.4.5.Molecular weight.....	56
2.5.4.6.Physical properties.....	56
2.5.4.7.Uses.....	56
2.5.5. (3-Aminopropyl) triethoxysilane	57
2.5.5.1.Chemical structure.....	57
2.5.5.2.Description.....	57
2.5.5.3.IUPAC name.....	57
2.5.5.4.Molecular formula.....	57
2.5.5.5.Molecular weight.....	57
2.5.5.6.Physical properties.....	57
2.5.5.7.Uses.....	57
2.6. Liposomes	57-58
2.6.1. Methods of preparation of liposomes	58
2.6.1.1.Thin film hydration method.....	58-59
2.6.1.2.Detergent removal method.....	59
2.6.1.3.Solvent injection method.....	59
2.6.1.3.1. Ethanol injection method.....	59
2.6.1.3.2. Ether injection method.....	60
2.6.1.4.Reverse phase evaporation method.....	60
2.6.1.5.Sonication method.....	60-61
2.6.1.6.Extrusion method.....	61
2.6.1.7.Homogenization.....	61-62
2.6.1.8. Novel methodologies.....	62
2.6.2. Drug profile	62
2.6.2.1. Chemical structure.....	62
2.6.2.2. Description.....	62-63
2.6.2.3. IUPAC name.....	63
2.6.2.4. Molecular formula.....	63
2.6.2.5. Molecular weight.....	63

2.6.2.6. Elimination half-life.....	63
2.6.2.7. Solubility.....	63
2.6.3. Cholesterol.....	63
2.6.3.1. Chemical structure.....	63
2.6.3.2. Description.....	63
2.6.3.3. IUPAC name.....	63
2.6.3.4. Molecular formula.....	63
2.6.3.5. Molecular weight.....	64
2.6.3.6. Physical properties.....	64
2.6.3.7. Solubility.....	64
2.6.3.8. Metabolism.....	64
2.6.4. Tocopheryl polyethylene glycol succinate	64
2.6.4.1. Chemical structure.....	64
2.6.4.2. Description.....	64
2.6.4.3. IUPAC name.....	65
2.6.4.4. Molecular formula.....	65
2.6.4.5. Molecular weight.....	65
2.6.5. Phosphatidylcholine	65
2.6.5.1. Chemical structure.....	65
2.6.5.2. Description.....	65
2.6.5.3. Molecular formula.....	65
2.6.5.4. Molecular weight.....	65
2.6.5.5. Physical properties.....	65
3. Formulation and optimization of abciximab coated mesoporous silica nanoparticles for improved antithrombotic activity.....	66-100
3.1. Objective.....	66
3.2 Plan of study.....	66-67
3.3. Material.....	67
3.4. Methods.....	67
3.4.1. Selection of CMAs and CQAs.....	67
3.4.1.1. Formulation optimization by Box-Behnken Design	68
3.4.2. Preparation of amino-functionalized MSN	68-69
3.4.3. Conjugation of ABX to MSN- NH ₂	69
3.4.4. Preparation of DiD dye loaded ABX coated MSN-NH ₂	69-70
3.4.5. Nanoparticle characterizations	71
3.4.5.1. Particle size, polydispersity and zeta potential.....	71
3.4.5.2. Transmission electron microscopy (TEM) analysis.....	71
3.4.5.3. Scanning electron microscopy (SEM) analysis.....	72
3.4.5.4. Surface chemistry	72
3.4.5.5. X-ray photoelectron spectroscopy (XPS) analysis.....	72
3.4.5.6. Thermogravimetric analysis (TGA).....	72-73
3.4.5.7. Degree of ABX conjugation.....	73
3.4.5.8. <i>In-vitro</i> characterization.....	73
3.4.5.8.1. <i>In-vitro</i> ABX release	73
3.4.5.8.2. <i>In-vitro</i> blood clot assay	74
3.4.5.9. <i>In-vitro</i> clot targeting efficiency.....	75

3.4.5.9.1. <i>In-vitro</i> platelet binding assay.....	75
3.4.5.9.2. Image-J analysis of confocal laser microscopic images.....	76
3.4.5.10. <i>In-vitro</i> safety assessment	77
3.4.5.10.1. Blood Smear.....	77
3.4.5.10.2. Hemolytic Assay.....	78-79
3.4.5.11. <i>In vivo</i> studies	79
3.4.5.11.1. Tail bleeding assay	79-80
3.4.5.11.2. Blood clotting time study.....	80
3.4.5.11.3. <i>In-vivo</i> FeCl ₃ induced model for thrombus formation by B.P. measurement.....	81-82
3.4.5.12. Statistical analysis.....	82
3.5. Results and discussion.....	84
3.5.1. Formulation optimization by Box-Behnken Design.....	84
3.5.1.1 Effect on particle size	84-85
3.5.1.2. Effect on PDI.....	85-86
3.5.2. Nanoparticles characterization	86
3.5.2.1. Size, polydispersity, and zeta potential of nanoparticles	86-87
3.5.2.2. TEM analysis	87
3.5.2.3. Scanning electron microscopy	87-88
3.5.2.4. Surface chemistry.....	88-89
3.5.2.5. XRD analysis.....	89
3.5.2.6. TGA study.....	89
3.5.2.7. Degree of ABX conjugation.....	90
3.5.2.8. <i>In-vitro</i> analysis	91
3.5.2.8.1. <i>In-vitro</i> ABX release	91
3.5.2.8.1. Blood clot assay	91
3.5.2.9. <i>In-vitro</i> clot targeting efficiency.....	92
3.5.2.9.1. <i>In-vitro</i> platelet binding assay	92-93
3.5.2.9.2. Image-J analysis of CLSM images.....	93
3.5.2.10 <i>In-vitro</i> safety assessment.....	94
3.5.2.10.1. Blood Smear.....	94
3.5.2.10.2. Hemolytic Assay.....	95
3.5.2.11. <i>In-vivo</i> studies.....	96
3.5.2.11.1. Tail bleeding assay.....	96
3.5.2.11.2. Blood clotting time study.....	96
3.5.2.11.3. <i>In-vivo</i> FeCl ₃ induced model for thrombus formation by B.P. measurement.....	97
3.6. Conclusion.....	98-100
4. GPIIb/IIIa receptor targeted rutin loaded liposomes for site-specific antithrombotic effect	101-148
4.1. Objective.....	101
4.2 Plan of study.....	101-102
4.3. Material.....	102
4.4. Methods.....	103
4.4.1. Selection of CMAs and CQAs.....	103

4.4.1.1. Formulation optimization by Box-Behnken design	103
4.4.2. Fabrication of nontargeted and targeted liposomes	104-105
4.4.3. Preparation of dye loaded liposomes.....	106
4.4.4. Evaluation of physicochemical characteristics.....	106
4.4.2.1. Particle size and polydispersity index (PI) determination .	106
4.4.2.2. ζ potential measurement	107
4.4.4.3. Encapsulation Efficiency.....	107
4.4.4.4. Scanning electron microscopy (SEM).....	107
4.4.4.5. Transmission electron microscopy (TEM).....	107-108
4.4.4.6. Degree of peptide conjugation.....	108
4.4.4.7. Surface chemistry.....	108
4.4.4.8. X-ray diffraction (XRD) analysis.....	108-109
4.4.4.9. Thermogravimetric analysis (TGA).....	109
4.4.5. <i>In-vitro</i> studies.....	109
4.4.5.1. <i>In-vitro</i> drug release study.....	109
4.4.5.2. Blood clot analysis.....	110
4.4.5.3. <i>In-vitro</i> coagulation aPTT and PT assay.....	110
4.4.5.4. Platelet aggregation study.....	111-112
4.4.5.5. Biocompatibility and safety evaluation.....	112
4.4.5.5.1.Blood smear analysis.....	112
4.4.5.5.2. Hemolytic assay.....	113
4.4.5.6. <i>In-vitro</i> cytotoxicity assay.....	113-114
4.4.5.7. <i>In-vitro</i> clot targeting efficiency by platelet binding.....	114
4.4.5.7.1. <i>In-vitro</i> imaging.....	114
4.4.5.7.2. ImageJ analysis of CLSM images.....	115
4.4.5.8. Stability studies.....	115
4.4.5.8.1. Stability study in serum.....	115
4.4.5.8.2. Storage stability study.....	115
4.4.6. <i>In-vivo</i> studies.....	115
4.4.6.1. Tail bleeding assay.....	115-116
4.4.6.2. Blood clotting time study.....	116
4.4.6.3. <i>In-vivo</i> FeCl ₃ model for thrombus formation by B.P measurement.....	116-117
4.4.6.4. Pharmacokinetics study.....	117-118
4.4.6.5. Toxicity assay.....	118
4.4.7. Statistical investigations.....	118-119
4.5. Results and discussion.....	119
4.5.1. Formulation optimization by Box-Behnken design.....	119
4.5.1.1. Effect on particle size.....	119-120
4.5.1.2. Effect on PDI.....	120-121
4.5.1.3. Effect on %EE.....	121-122
4.5.2. Evaluation of physicochemical characteristics.....	123
4.5.2.1. Particle size, PI, and ζ potential determination.....	123
4.5.2.2. Encapsulation efficiency.....	123-124
4.5.2.3. SEM and TEM	124-125
4.5.2.4. Bradford assay.....	125-126

4.5.2.5. Surface chemistry.....	126-127
4.5.2.6. XRD studies.....	127-128
4.5.2.7. TGA studies.....	128-129
4.5.3. <i>In-vitro</i> studies.....	129
4.5.3.1. <i>In-vitro</i> drug release study.....	129-130
4.5.3.2. Blood clot analysis	130-131
4.5.3.3. <i>In-vitro</i> coagulation aPTT and PT assay.....	131-132
4.5.3.4. Platelet aggregation studies.....	132-133
4.5.3.5. Biocompatibility and safety evaluation.....	133
4.5.3.5.1. Blood Smear.....	133-134
4.5.3.5.2. Hemolytic Assay.....	134-135
4.5.3.6. <i>In-vitro</i> cytotoxicity assay.....	135-136
4.5.3.7. <i>In-vitro</i> clot targeting efficiency by platelet binding...	137
4.5.3.7.1. <i>In-vitro</i> imaging.....	137
4.5.3.7.2. Image-J analysis of CLSM images.....	137-138
4.5.3.8. Stability study.....	139
4.5.3.8.1. Serum stability study.....	139
4.5.3.8.2. Storage stability study.....	139-140
4.5.4. <i>In-vivo</i> studies.....	140
4.5.4.1. Tail bleeding assay.....	140-141
4.5.4.2. Blood clotting time study.....	141
4.5.4.3.FeCl ₃ model for thrombus formation by B.P. measurement.....	141-144
4.5.4.4. Pharmacokinetic study.....	144-146
4.5.4.5. Toxicity assay.....	146-147
4.6. Conclusion.....	147-148
5. Summary and conclusions.....	149-155
6. References.....	156-185
7. Publications.....	186-187
8. Curriculum Vitae.....	188-193