

## CONTENTS

List of contents	Page No.
<b>PART I: INTRODUCTION, LITERATURE REVIEW AND PROFILE OF DRUGS</b>	<b>1-82</b>
<b>Chapter 1 AIM OF PRESENT WORK</b>	<b>1-3</b>
1.1 Introduction	1
1.2 Proposed plan of work	2
<b>Chapter 2 LITERATURE REVIEW</b>	<b>4-54</b>
<b>2.1 Cancer</b>	<b>4</b>
2.1.1 General	4
2.1.2 Costs for Cancers	4
2.1.3 Classification of Cancer	6
<b>2.2 Prostate Cancer</b>	<b>6</b>
2.2.1 Introduction	6
2.2.2 Prostate Cancer Statistics	6
2.2.3 Causes and Symptoms of Prostate Cancer	7
2.2.4 Prostate cancer treatment options	8
<b>2.3 Leukemia</b>	<b>10</b>
2.3.1 Introduction	10
2.3.2 Classification of leukemia	11
2.3.3 Causes and symptoms of leukemia	11
2.3.4 Treatments for leukemia	12
2.3.4.1 Chemotherapy Drugs	12
2.3.5 Precautions during treatment	13
	13
<b>2.4 Liposomes</b>	<b>15</b>
2.4.1 Introduction	15
2.4.2 Classification of liposomes	16
2.4.2.1. On the basis of size	16
2.4.2.2. On the basis of composition	17
2.4.3 Methods of preparation	19
2.4.4 Disadvantages of conventional liposomes	20
	20
<b>2.5 Stealth liposomes or Long circulating liposomes (LCL)</b>	<b>21</b>
2.5.1 Introduction	21
2.5.2 Composition of stealth liposomes	21
2.5.2.1 Polyethylene glycols	21
	21

2.5.2.2 Gangliosides and glycolipids	22
2.5.2.3 Synthetic phospholipids	22
2.5.3 Effect of PEG grafting on protein adsorption and cell adhesion	24
2.5.4 Characterization of stealth liposomes	25
2.5.5 Applications of liposomes in drug delivery	26
2.5.5.1. Formulation aid	26
2.5.5.2 Intracellular drug delivery	28
2.5.5.3 Sustained release drug delivery	28
2.5.5.4. Gene therapy	28
2.5.5.5. Site-avoidance delivery	29
2.5.5.6. Site-specific targeting	29
2.5.5.6.1. Passive targeting.	29
2.5.5.6.2. Active targeting	30
2.5.5.7. Intraperitoneal administration	31
2.5.5.8. Immunological adjuvants in vaccines	31
2.5.6. Limitations of liposome technology	32
2.5.6.1. Stability	32
2.5.6.2. Sterilization	33
2.5.6.3. Encapsulation efficiency	33
2.5.6.4. Active targeting	33
2.5.6.5. Gene therapy	34
2.5.6.6. Lysosomal degradation	34
<b>2.6 Microbubbles</b>	<b>35</b>
2.6.1 Introduction	35
2.6.2 Background	35
2.6.3 Composition of microbubbles	37
2.6.4 Types or classification of microbubbles	39
2.6.5 Methods of preparation of microbubbles	40
2.6.5.1 Protein coated microbubbles	40
2.6.5.2 Surfactant based microbubbles	41
2.6.5.3 Polymer based gas-filled micro particles	42
2.6.5.4 Perfluorocarbon-exposed dextrose albumin (PESDA) microbubbles	43
2.6.5.5 Lipid coated microbubbles	43
2.6.5.6 Adenovirus Microbubbles	44
2.6.5.7 Preparation of ST68 microbubbles	45
2.6.5.8 Acoustically active liposomes (AALs)	46
2.6.6 Characterization of Microbubbles	46
2.6.6.1 Microbubble size, size distribution and concentration	46
2.6.6.2 Air or gas content measurement	46
2.6.6.3 Surface morphology by SEM/TEM studies	47
2.6.6.4 Echogenic properties by ultrasound attenuation measurements	47
2.6.6.5 Microbubble ultrasound sensitivity determination	47
2.6.6.6 Inertial cavitation threshold measurements	48
2.6.6.7 In vitro measurement of acoustic properties	49
2.6.6.8 In vitro drug partitioning measurements	50

2.6.7 Mechanisms for target drug delivery using microbubbles	50
2.6.8 Mechanism for Microbubble application in diagnosis	51
2.6.9 Application of microbubbles	52
2.6.9.1 Diagnostic applications	52
2.6.9.2 Microbubbles in therapy—ultrasound-assisted drug and gene delivery	53
<b>Chapter 3 PROFILE OF DRUGS</b>	<b>55-82</b>
<b>3.1 Flutamide</b>	<b>55</b>
3.1.1 Nomenclature	55
3.1.1.1 Chemical Names	55
3.1.1.2 Nonproprietary Names	55
3.1.1.3 Proprietary Names	55
3.1.2 Formulae	55
3.1.2.1 Empirical	55
3.1.2.2 Structural	55
3.1.3 Molecular Weight	55
3.1.4 CAS Number	55
3.1.5 Appearance	55
3.1.6 Uses and Applications	56
3.1.7 Physical Properties	56
3.1.7.1 Particle Morphology	56
3.1.7.2 Crystallographic Properties	56
3.1.7.2.1 Single Crystal Structure	56
3.1.7.2.2 Polymorphism	58
3.1.7.3 Thermal Methods of analysis	58
3.1.7.3.1 Melting Behavior	58
3.1.7.3.2 Differential Scanning Calorimetry	59
3.1.7.3.3 Thermogravimetric Analysis	59
3.1.7.4 Hygroscopicity	59
3.1.7.5 Solubility Characteristics	60
3.1.7.6 Partition Coefficients	60
3.1.7.7 Ionization Constants	61
3.1.7.8 Spectroscopy	61
3.1.7.8.1 UV/VIS Spectroscopy	61
3.1.7.8.2 Vibrational Spectroscopy	62
3.1.8 Methods of Analysis	63
3.1.8.1 Compendial Tests	63
3.1.8.1.1 Identification	63
3.1.8.1.2 Melting Range	63
3.1.8.1.3 Loss on Drying	63
3.1.8.1.4 Residue on Ignition	63
3.1.8.1.5 Heavy Metals	63
3.1.8.1.6 Chromatographic Purity	63
3.1.8.1.7 Assay	64
3.1.8.1.8 Chromatographic System	64
3.1.8.2 Elemental Analysis	64
3.1.8.3 Electrochemical Analysis	65
3.1.8.4 Spectrophotometric Methods of Analysis	65

3.1.8.5 Chromatographic Methods of Analysis	65
3.1.8.5.1 Thin Layer Chromatography	65
3.1.8.5.2 Gas Chromatography	66
3.1.8.5.3 High Performance Liquid Chromatography	66
3.1.8.6 Determination in Body Fluids and Tissues	67
3.1.9. Stability	68
3.1.9.1 Solid-State Stability	68
3.1.9.2 Solution-Phase Stability	68
3.1.10 Clinical Pharmacology	68
3.1.10.1 General	68
3.1.10.2 Dosage and administration	68
3.1.10.2.1 How Supplied	68
3.1.10.3 Pharmacokinetics	69
3.1.10.3.1 Absorption	69
3.1.10.3.2 Distribution	69
3.1.10.3.3 Metabolism	69
3.1.10.3.4 Excretion	70
3.1.10.4 Indications	70
3.1.10.4.1 Stage B <sub>2</sub> -C Prostatic Carcinoma	70
3.1.10.4.2 Stage D <sub>2</sub> Metastatic Carcinoma	70
3.1.10.5 Side effects	70
3.1.10.5.1 Hepatic Injury	70
3.1.10.5.2 Renal Impairment	71
3.1.10.5.3 Central Nervous System	71
3.1.10.5.4 Gastrointestinal System	71
3.1.10.5.5 Hematopoietic System	71
3.1.10.5.6 Skin	71
3.1.10.5.7 Others	71
3.1.10.6 Interactions	72
3.1.10.7 Laboratory Tests	72
<b>3.2 6-Mercaptopurine</b>	73
3.2.1 Description	73
3.2.1.1 Name, Formula, Molecular Weight	73
3.2.1.2 Appearance, Color, Odor	73
3.2.2 Physical Properties	73
3.2.2.1 Infrared Spectrum	73
3.2.2.2 Ultraviolet Spectrum	73
3.2.2.3 Melting Point	73
3.2.2.4 Solubility	75
3.2.2.5 Dissociation Constant	75
3.2.3 Synthesis	75
3.2.4 Stability	75
3.2.5 Methods of Analysis	75
3.2.5.1 Elemental Analysis	75
3.2.5.2 Nonaqueous Titrimetric Analysis	75
3.2.5.3 Spectrophotometric Analysis	75
3.2.5.4 Polarography	76
3.2.5.5 Mass Spectrometry	76
3.2.5.6 Chromatography	76

3.2.5.6.1 High Performance Liquid Chromatography	76
3.2.5.6.2 Column Chromatography	76
3.2.5.6.3 Gas Chromatography	77
3.2.5.6.4 Thin Layer Chromatography	77
<b>3.2.6 Clinical Pharmacology</b>	<b>77</b>
3.2.6.1 Mechanism of action	77
3.2.6.2 Pharmacokinetics	77
3.2.6.2.1 Oral Absorption	77
3.2.6.2.2 Tissue distribution	78
3.2.6.2.3 Metabolism	78
3.2.6.2.4 Excretion	79
3.2.6.3 Indication and Usage	79
3.2.6.4 Adverse effects	79
3.2.6.5 Dosage	81
3.2.6.5.1 Adults	81
3.2.6.5.2 Children	81
3.2.6.6 Special Precautions	81
3.2.6.7 Drug-drug interactions	82

<b>PART II: METHOD DEVELOPMENT, PREPARATION AND COMPARATIVE EVALUATION OF STEALTH LIPOSOMES AND MICROBUBBLES FOR THERAPEUTIC PURPOSE</b>	<b>83-232</b>
<b>Chapter 4 ANALYTICAL METHODS</b>	<b>83-107</b>
<b>Introduction</b>	<b>83</b>
<b>4.1 Estimation of phosphatidylcholine in liposomes</b>	<b>83</b>
4.1.1 Solutions	83
4.1.2 Procedure for calibration curve	83
4.1.3 Estimation of phosphatidylcholine from liposomes/supernatant	84
4.1.4 Stability and selectivity	84
<b>4.2 Estimation of cholesterol in liposomes</b>	<b>85</b>
4.2.1 Solutions	85
4.2.2 Procedure for calibration curve	86
4.2.3 Estimation of cholesterol from liposomes/supernatant:	86
4.2.4 Stability and selectivity	86
<b>4.3 Estimation of polyethylene glycol derivatives in liposomes</b>	<b>87</b>
4.3.1 Solutions	87
4.3.2 Procedure for calibration curve	88
4.3.3 Estimation of polyethylene glycol derivatives from liposomes/supernatant	88
4.3.4 Stability and selectivity	88
<b>4.4 Estimation of Flutamide</b>	<b>90</b>
4.4.1 Spectrophotometric estimation of flutamide	90
4.4.1.1 Chemicals and Reagents	90
4.4.1.2 Calibration curve	90
4.4.1.3 Estimation of flutamide from liposomes and supernatant	91
4.4.2 High-performance liquid chromatographic estimation of flutamide	92
4.4.2.1 Introduction	92
4.4.2.2 Experimental	92
4.4.2.2.1 Apparatus	92
4.4.2.2.2 Chromatographic conditions	92
4.4.2.2.3 Preparation of standards	93
4.4.2.2.4 Blood collection	93
4.4.2.2.5 Validation procedures	93
4.4.2.2.6 Determination of FLT in rat plasma samples	94
4.4.2.3 Results and Discussion	95

4.4.2.3.1 Characteristics of the chromatographic peak	95
4.4.2.3.2 Linearity	96
4.4.2.3.3 Accuracy, Precision and Robustness	96
4.4.2.3.4 Application to rat plasma samples	97
4.4.2.4 Summary and Conclusion	98
<b>4.5 Estimation of 6-Mercaptopurine</b>	99
4.5.1 Spectrophotometric estimation of 6-Mercaptopurine	99
4.5.1.1 Chemicals and Reagents	99
4.5.1.2 Calibration curve	99
4.5.1.3 Estimation of 6-MP from liposomes and supernatant	100
4.5.2 High-performance liquid chromatographic estimation of 6-Mercaptopurine	100
4.5.2.1 Introduction	100
4.5.2.2 Experimental	101
4.5.2.2.1 Preparation of Stock and Standard Solutions	101
4.5.2.2.2 Blood collection and tissue preparation	102
4.5.2.2.3 Calibration of 6-MP in plasma	102
4.5.2.2.4 Calibration of 6-MP in various tissues	102
4.5.2.2.5 HPLC system and Chromatographic conditions	103
4.5.2.3 Results and Discussion	103
4.5.2.3.1 HPLC method development	103
4.5.2.3.2 Estimation of 6-MP in plasma	104
4.5.2.3.3 Estimation of 6-MP in various tissues	106
4.5.2.4 Summary and Conclusion	107
<b>Chapter 5 PREPARATION OF LIPOSOMES AND MICROBUBBLES</b>	108-150
<b>5.1 Introduction</b>	108
5.1.1 General	108
5.1.2 Drugs	108
5.1.3 Materials	108
5.1.4 Apparatus	109
5.1.5 Solutions	109
<b>5.2 Preparation of Flutamide liposomes</b>	109
5.2.1 Introduction	109
5.2.2 Experimental Design	110
5.2.3 Preparation of Liposomes	110
5.2.4 Statistical Analysis	111
5.2.5 Characterization of Liposomes	112
5.2.5.1 % Encapsulation Efficiency	112
5.2.5.2 Photomicrography	113
5.2.6 Results And Discussion	114
5.2.7 Checkpoint Experiment	117
5.2.8 Conclusion	118

<b>5.3 Preparation of Flutamide stealth liposomes using poly ethylene glycol derivatized phosphatidylethanolamine (mPEG<sub>2000</sub>-PE)</b>	119
5.3.1 Introduction	119
5.3.2 Experimental	119
5.3.2.1 Synthesis of methoxy polyethylene glycol 2000 activated with cyanuric chloride (mPEG <sub>2000</sub> -CC)	119
5.3.2.2 Synthesis of methoxy polyethylene glycol 2000 activated with cyanuric chloride-phosphatidylethanolamine conjugate (mPEG <sub>2000</sub> -CC-PE)	122
5.3.2.3 Preparation of flutamide stealth liposomes using mPEG <sub>2000</sub> -CC-PE	126
5.3.2.4 Electrolyte Induced Flocculation Test	126
5.3.3 Results And Discussion	127
5.3.3.1 % Entrapment Efficiency	127
5.3.3.2 Electrolyte Induced Flocculation Test	127
5.3.4 Conclusion	129
<b>5.4 Preparation of 6-Mercaptopurine liposomes</b>	130
5.4.1 Introduction	130
5.4.2 Experimental	130
5.4.2.1 Statistical Design	130
5.4.2.2 Preparation of liposomes	131
5.4.2.3 Statistical Analysis	131
5.4.2.4 Characterization of liposomes	134
5.4.2.4.1 % Encapsulation Efficiency	134
5.4.2.4.2 Photomicrography	134
5.4.3 Results And Discussion	134
5.4.4 Conclusion	137
<b>5.5 Preparation of 6-Mercaptopurine stealth liposomes using mPEG<sub>2000</sub>-CC-PE</b>	137
5.5.1 Experimental	137
5.5.2 Results And Discussion	138
5.5.2.1 % Entrapment Efficiency	138
5.5.2.2 Electrolyte Induced Flocculation Test	138
5.5.3 Conclusion	140
<b>5.6 Preparation of Microbubbles</b>	141
5.6.1 Introduction	141
5.6.2 Experimental	141
5.6.2.1 Materials	141
5.6.2.2 Apparatus	141
5.6.2.3 Methods of preparation for Flutamide and 6-Mercaptopurine Microbubbles	142
5.6.2.3.1 Method 1: vortexing with freeze and	142

thaw cycles method 5.6.2.3.2 Method 2: modified double emulsion (W <sub>1</sub> /O <sub>1</sub> /W <sub>2</sub> ) solvent evaporation technique 5.6.2.3.3 Method 3: mixing cum sonication technique <b>5.6.3 Results and Discussion</b> 5.6.3.1 Flutamide Microbubbles (FLT-MBs) 5.6.3.2 6-Mercaptopurine Microbubbles (6-MP MBs) 5.6.3.3 Flutamide Gas Filled Microparticles (FLT-GFMs) 5.6.3.4 6-Mercaptopurine Gas Filled Microparticles (6-MP-GFMs) 5.6.3.5 Acoustically Active Liposomes (AALs) of Flutamide and 6-Mercaptopurine <b>5.6.4 Conclusion</b>	142 143 144 144 146 147 148 149 150
<b>Chapter 6: CHARACTERIZATION OF LIPOSOMES AND MICROBUBBLES</b>	
<b>6.1 Introduction</b>	151
<b>6.2 Experimental</b>	151
6.2.1 Drugs and reagents	151
6.2.2 Apparatus	151
6.2.3 Characterization of liposomes	152
6.2.3.1 % Entrapment efficiency	152
6.2.3.2 Electrolyte induced flocculation test	152
6.2.3.3 Morphology of liposomes by photomicrography	152
6.2.3.4 Particle size and particle size distribution	152
6.2.3.5 Transmission electron microscopy	153
6.2.3.6 Zeta potential measurements	153
6.2.3.7 Differential scanning calorimetry	154
6.2.3.8 Lamellarity by <sup>31</sup> P-NMR studies	154
<b>6.3 Results and discussion</b>	155
6.2.3.1 % Entrapment efficiency	155
6.2.3.2 Electrolyte induced flocculation test	156
6.2.3.3 Morphology of liposomes by photomicrography	156
6.2.3.4 Particle size and particle size distribution	156
6.2.3.5 Transmission electron microscopy	159
6.2.3.6 Zeta potential measurements	159
6.2.3.7 Differential scanning calorimetry	160
6.2.3.8 Lamellarity by <sup>31</sup> P-NMR studies	161
<b>6.4 Conclusion</b>	164
<b>6.5 Characterization of microbubbles</b>	165

<b>6.5.1 Experimental</b>	165
6.5.1.1 Bubble size distribution and bubble number	165
6.5.1.2 Surface morphology by photomicrography	165
6.5.1.3 Gas content by densitometry	165
6.5.1.4 Effect of sonication and centrifugation	166
6.5.1.5 In vitro drug partitioning studies	166
6.5.1.6 Effect of different frequency ultrasound transducers	167
<b>6.5.2 Results and Discussion</b>	167
6.5.2.1 Bubble size distribution and bubble number	167
6.5.2.2 Surface morphology by photomicrography	170
6.5.2.3 Gas content by densitometry	172
6.5.2.4 Effect of sonication on bubble size and number	172
6.5.2.5 Effect of centrifugation on bubble size	174
6.5.2.6 In vitro drug partitioning studies	175
6.5.2.7 Effect of different frequency ultrasound transducers	175
<b>Chapter 7: IN VITRO DRUG RELEASE STUDIES FROM LIPOSOMES AND MICROBUBBLES</b>	179-184
<b>7.1 Introduction</b>	179
<b>7.2 Experimental</b>	180
7.2.1 Solution	180
7.2.2 Apparatus	180
7.2.3 In vitro drug release from liposomes and microbubbles	180
<b>7.3 Results And Discussion</b>	180
7.3.1 In vitro drug release from liposomes	180
7.3.2 In vitro drug release from microbubbles	183
<b>7.4 Conclusion</b>	185
<b>Chapter 8: STABILITY STUDIES OF LIPOSOMES AND MICROBUBBLES</b>	187-198
<b>8.1 Introduction</b>	187
8.1.1 General	187
8.1.2 Stability testing and lyophilization	188
<b>8.2 Experimental</b>	188
8.2.1 Reagents	188
8.2.2 Apparatus	188
8.2.3 Solutions	189
8.2.4 Lyophilization of FLT and 6-MP conventional and stealth liposomes	189
8.2.5 Stability studies of FLT and 6-MP conventional and	189

stealth liposomes	
<b>8.3 Results and discussion</b>	190
8.3.1 Lyophilization of FLT and 6-MP conventional and stealth liposomes	190
8.3.2 Stability studies of FLT and 6-MP conventional and stealth liposomes	191
8.3.3 Particle size and stability studies	194
8.3.4 Steric stabilization and stability studies	195
8.3.5 Stability studies of microbubbles	196
<b>8.4 Conclusion</b>	197
<b>Chapter 9: IN VITRO CELL CYTOTOXICITY STUDIES</b>	199-214
<b>9.1 Introduction</b>	199
9.1.1 Methods for measurement of cytotoxicity	199
9.1.2 Principle of MTT assay	200
9.1.3 Advantages of MTT method	200
<b>9.2 Experimental</b>	201
9.2.1 Cell lines	201
9.2.2 Solutions	201
9.2.2.1 MTT solution	201
9.2.2.2 Solution of Flutamide (FLT) and its liposomal formulations	201
9.2.2.3 Solution of 6-Mercaptopurine (6-MP) and its liposomal formulations	201
9.2.2.4 MTT Assay for liposomal formulations	202
9.2.2.5 Microbubble formulations containing Flutamide (FLT) and 6-Mercaptopurine (6-MP)	202
9.2.2.6 MTT Assay for microbubbles	203
<b>9.3 Results and Discussion</b>	203
9.3.1 Flutamide and its liposomes	204
9.3.2 6-Mercaptopurine and its liposomes	206
9.3.3 Flutamide microbubbles	208
9.3.4 6-Mercaptopurine microbubbles	212
<b>9.4 Conclusion</b>	214

<b>Chapter 10: PHARMACOKINETIC AND BIODISTRIBUTION STUDIES</b>	215-227
<b>10.1 Introduction</b>	215
<b>10.2 Experimental</b>	215
10.2.1 Selection of animals	215
10.2.2 Pharmacokinetic studies	215
10.2.2.1 Pharmacokinetic studies of pure flutamide and its formulations	215
10.2.2.2 Pharmacokinetic studies of pure 6-Mercaptopurine and its formulations	216
10.2.3 Biodistribution studies	217
10.2.3.1 Biodistribution studies of flutamide and its formulations	217
10.2.3.2 Biodistribution studies of 6-Mercaptopurine and formulations	217
<b>10.3 Results And Discussion</b>	218
10.3.1 Pharmacokinetic studies of pure flutamide and its liposomal formulations	218
10.3.2 Pharmacokinetic studies of pure 6-Mercaptopurine and its liposomal formulations	220
10.3.3 Biodistribution studies of flutamide, its liposomes and microbubble formulations	223
10.3.4 Biodistribution studies of 6-MP and its liposomal formulations	225
<b>10.4 Conclusion</b>	227
<b>Chapter 11: HEPATOTOXICITY STUDIES</b>	228-235
<b>11.1 Introduction</b>	228
11.1.1 General	228
11.1.2 Drug related side effects	228
11.1.3 Laboratory Tests	229
<b>11.2 Experimental</b>	229
11.2.1 Selection of animals	229
11.2.2 Hepatotoxicity studies of FLT and its preparations	229
11.2.2.1 Histopathological studies and Biochemical analysis	229
11.2.3 Hepatotoxicity studies of 6-MP and its preparations	230
11.2.3.1 Histopathological studies and Biochemical analysis	230
<b>11.3 Results And Discussion</b>	230
11.3.1 Histopathological studies and Biochemical analysis of	230

FLT and its preparations	
11.3.2 Histopathological studies and Biochemical analysis of 6-Mercaptopurine and its preparations	233
<b>11.4 Conclusion</b>	235

<b>PART III: COMPARATIVE EVALUATION OF STEALTH LIPOSOMES AND MICROBUBBLES IN DIAGNOSIS</b>	236-248
<b>CHAPTER 12: APPLICATION OF LIPOSOMES AND MICROBUBBLES IN DIAGNOSIS</b>	236-248
<b>12.1 Introduction (Diagnostic Imaging)</b>	236
12.1.1 Imaging Modalities: Overview	236
12.1.1.1 X-Ray	236
12.1.1.2 CT [computer (assisted) tomography]	236
12.1.1.3 Nuclear Medicine	236
12.1.1.4 PET (Positron Emission Tomography)	236
12.1.1.5 SPECT (Single Photon Emission Computed Tomography)	237
12.1.1.6 Ultrasound	237
12.1.1.7 Optical Imaging	237
12.1.1.8 MRI (magnetic resonance imaging)	237
12.1.2 Ultrasound	238
<b>12.2 Experimental</b>	239
12.2.1 Selection of animals	239
12.2.2 Materials	240
12.2.3 Apparatus	240
12.2.4 Preparation of ultrasound contrast agents	240
12.2.4.1 Dye solution	240
12.2.4.2 Liposome based ultrasound contrast agents	240
12.2.4.3 Microbubble based ultrasound contrast agents	240
12.2.5 Sonography and Doppler studies	240
<b>12.3 Results and Discussion</b>	241
12.3.1 Sonography studies	241
12.3.2 Doppler studies	244
<b>12.4 Conclusion</b>	248
<b>SUMMARY AND CONCLUSION</b>	249-265
<b>REFERENCES</b>	266-304
<b>LIST OF PUBLICATIONS AND PRESENTATIONS</b>	-