
TABLE OF CONTENTS

CHAPTER-1: INTRODUCTION.....	8-46
1.1 Cancer:.....	8
1.2 Epidemiology:	8
1.3 Pathogenesis of prostate cancer.....	10
1.4 Symptoms associated with the disease	11
1.5 Diagnosis and management of prostate cancer	11
1.6 Current challenges in prostate cancer diagnosis and treatment.....	12
1.7 Recent scenario.....	14
1.8 Anticancer drugs.....	16
1.9 Nanotechnology.....	17
1.10 Mesoporous silica nanoparticles.....	18
1.11 History associated with MSNs	19
1.12 Synthesis of Mesoporous silica Nanoparticles	21
1.12.1 Sol-gel synthesis.....	21
1.12.2 Hydrothermal synthesis	21
1.12.3 Microwave synthesis	22
1.12.4 Template synthesis	22
1.13 Characterization of MSNs	22
1.14 Drug loading	23
1.15 Surface functionalization of MSNs	23
1.16 Efficiency of surface functionalization	26
1.17 Targeted drug delivery using MSN	27
1.17.1 MSNs in cancer treatment:	27
1.17.2 Passive targeting in cancer	27
1.17.3 Active targeting	28
1.18 Biocompatibility and biodegradation of MSNs.....	28

1.19 Recent advances and other applications of MSNs	30
1.20 Challenges of MSN application in cancer therapy and Present scenario:	31
1.21 References	34
CHAPTER-2: Literature review	47-62
2.1 Introduction	47
2.2 Selection of active pharmaceutical ingredients	49
2.2.1 Etoposide (ETO).....	49
2.2.2 Bicalutamide (BIC)	52
2.3 Need for the study	54
2.4 References	56
CHAPTER-3: AIMS AND OBJECTIVES	63-67
3.1 Aims.....	63
3.2 Objectives	63
3.3 Work plan	64
3.4 Hypothesis	66
3.5 Expected outcomes	67
CHAPTER-4: ANALYTICAL METHOD DEVELOPMENT AND VALIDATION	68-99
4.1 Introduction	68
4.2 Materials and Methods	68
4.3 Etoposide	69
4.3.1 UV spectrophotometric method development for ETO	69
4.3.2 Spectrofluorometric method development for ETO.....	70
4.3.3 RP-HPLC-FL method development for ETO.....	72
4.3.4 RP-HPLC-FL bioanalytical method development for ETO	74
4.4 Result and discussion for Etoposide.....	76
4.4.1 UV spectrophotometric method development.....	76
4.4.2 Spectrofluorimetric method development	77

4.4.3 RP-HPLC-FL method development for ETO.....	78
4.4.4. RP-HPLC-FL bioanalytical method development for ETO.....	80
4.5 Bicalutamide.....	85
4.5.1 UV spectrophotometric method development for BIC	85
4.5.2 Spectrofluorimetric method development for BIC.....	85
4.5.3 RP-HPLC-FL method development for BIC.....	86
4.5.4 RP-HPLC-FL bioanalytical method development for BIC	87
4.6 Result and discussion for Bicalutamide	88
4.6.1 UV spectrophotometric method development for BIC	88
4.6.2 Spectrofluorometric method development for BIC.....	90
4.6.3 RP-HPLC-FL method development for BIC.....	91
4.6.4 RP-HPLC-FL Bioanalytical method development.....	92
4.7 Summary.....	97
4.8 References	98
CHAPTER-5: FORMULATION DEVELOPMENT AND CHARACTERIZATION 100-	
108	
5.1 Materials.....	100
5.2 Methods	102
5.2.1 Synthesis of bare MCM-41 type of MSNs	102
5.2.2 Surface functionalization of MCM-41 NPs.....	102
5.2.3 FITC Labelling of MSNs.....	103
5.4. Solid state evaluation of bare and surface functionalized MSNs.....	104
5.4.1 Fourier Transform Infra-red (FT-IR) analysis.....	104
5.4.2 Differential scanning calorimetry (DSC) analysis:	105
5.4.3 Thermogravimetric analysis	105
5.4.4 Wide angle X-Ray diffraction analysis (W-XRD)	105
5.4.5 Low angle X-Ray diffraction analysis (L-XRD).....	106
5.4.6 Zeta potential and size determination:.....	106

5.4.7 Nitrogen sorption analysis:	106
5.4.8 SEM and TEM analysis:	107
5.4.9 Elemental detection and quantification of surface moiety	107
5.5 References:	108
CHAPTER-6: ETOPOSIDE.....	109-172
6.1 Introduction:	109
6.2 Materials and methods:.....	111
6.2.1 Chemicals and reagents	111
6.2.3 ETO loading into mesoporous network.....	113
6.2.3 In vitro release study:	113
6.2.4 In vitro cytotoxicity study	116
6.2.5 Caco-2 monolayer cell line permeability study:.....	118
6.2.6 In vitro cellular uptake study	119
6.2.7 Evaluation of cell death mechanisms by apoptosis assay	120
6.2.8 Haemolysis study.....	120
6.2.9 In vivo pharmacokinetic study	121
6.2.10 Statistical analysis	123
6.2.11 Stability study of mesoporous silica nanoparticles	123
6.3 Results and discussion.....	123
6.3.1 Solid state evaluation.....	123
6.3.1.1 Fourier Transform-Infra Red (FT-IR) spectroscopy studies	123
6.3.1.2 Differential scanning calorimetry (DSC) analysis	126
6.3.1.3 Thermogravimetric analysis	127
6.3.1.4 Wide angle X-Ray diffraction analysis (W-XRD).....	129
6.3.1.5 Low angle X-Ray diffraction.....	130
6.3.1.6 Zeta potential and size determination:.....	131
6.3.1.7 Nitrogen sorption analysis:.....	133

6.3.1.8 SEM and TEM analysis:.....	135
6.3.1.9 Elemental detection and quantification of surface moiety	137
6.3.2 Estimation of drug loading efficiency	138
6.3.3 In vitro release study:	139
6.3.4 In vitro cytotoxicity study	150
6.3.5 Caco-2 monolayer cell line permeability study:.....	153
6.3.6 In vitro cellular uptake study	155
6.3.6.2 Intracellular qualitative uptake study by confocal microscopy	155
6.3.6.3 Intracellular quantitative uptake study by Flow cytometry	156
6.3.7 Evaluation of cell death mechanisms by apoptosis assay	159
6.3.8 Haemolysis study.....	160
6.3.9 In vivo pharmacokinetic study	161
6.3.9.1 Pharmacokinetic study for oral formulation.....	161
6.3.9.2 Pharmacokinetic study for parenteral formulation	162
6.3.10 Stability study of mesoporous silica nanoparticles	165
6.4 Conclusion.....	167
6.5 References:	168
CHAPTER-7: BICALUTAMIDE.....	173-215
7.1 Introduction:	173
7.2 Materials and methods:.....	174
7.2.1 Chemicals and reagents	174
7.2.2 Synthesis of bare and functionalised MSNs	176
7.2.3 BIC loading	176
7.2.4 Formulation development.....	176
7.2.5 In vitro study.....	177
7.2.6 In vitro cytotoxicity study	179
7.2.7 Caco-2 monolayer cell line permeability study:.....	180

7.2.8 In vitro cellular uptake study	181
7.2.9 Evaluation of cell death mechanisms by apoptosis assay	181
7.2.10 Haemolysis study.....	182
7.2.11 In vivo pharmacokinetic study	182
7.2.12 Statistical analysis	185
7.2.13 Stability study of mesoporous silica nanoparticles	185
7.3 Results and discussion.....	185
7.3.1 Solid state evaluation.....	185
7.3.1.1 Fourier Transform-Infra Red (FT-IR) spectroscopy studies	185
7.3.1.2 Differential scanning calorimetry (DSC) analysis	187
7.3.1.3 Thermogravimetric analysis	188
7.3.1.4 Wide angle X-Ray diffraction analysis (W-XRD)	189
7.3.1.5 Low angle X-Ray diffraction.....	190
7.3.1.6 Zeta potential and size determination:.....	191
7.3.1.7 Nitrogen sorption analysis:.....	192
7.3.2 Estimation of drug loading efficiency	193
7.3.3 In vitro release study:	194
7.3.4 In vitro cytotoxicity study	202
7.3.5 Caco-2 monolayer cell line permeability study:.....	204
7.3.6 In vitro cellular uptake study	205
7.3.7 Evaluation of cell death mechanisms by apoptosis assay	206
7.3.8 Haemolysis study.....	206
7.3.9 In vivo pharmacokinetic study	207
7.3.9.1 Pharmacokinetic study for oral formulation.....	207
7.3.9.2 Pharmacokinetic study for parenteral formulation	208
7.3.10 Stability study of mesoporous silica nanoparticles	211
7.4 Conclusion.....	213

7.5 References:	214
CHAPTER-8: SUMMARY.....	216-223
8.1 Summary for ETO	217
8.2 Summary for BIC	221
CHAPTER-9: LIST OF PUBLICATIONS.....	224-225