
Contents

	List of abbreviations	i
	List of Figures.....	iii
	List of Tables.....	v
1	Introduction.....	3
1.1	Alzheimer’s Disease (AD).....	5
1.2	Diagnostics for AD: labelling and imaging.....	5
1.3	Pathogenesis of AD	5
1.3.1	Amyloid β and AD pathogenesis	6
1.3.2	Hyperphosphorylation of τ and Microglial infiltration leading to neurodegeneration.....	7
1.4	Nanotechnology as a therapeutic tool for drug delivery across the BBB	9
1.5	Therapeutics in AD.....	10
1.5.1	Modulating neurotransmission	10
1.5.2	Cholinesterase system.....	10
1.5.3	N-methyl D-aspartate antagonism	13
1.5.4	Amyloid Targeted strategies.....	14
1.5.5	Modulating A β transport.....	15
1.5.6	Decreasing A β aggregation by passive immunization therapy.....	16
1.6	Memantine for the treatment of moderate to severe Alzheimer’s	17
1.7	Self-Assembled Nanoscaffolds.....	17
2	Literature Review	21
2.1	Literature Review related to Memantine	21
2.2	Literature review on the development of memantine-loaded nanocarriers.	22
2.3	Literature Review: Self-assembled nanocarriers	24
2.4	Mechanism of action of self-assembled PLGA nanoscaffolds.....	26
2.5	Selection of Intrathecal route over oral route of administration.....	27
3	Drug and Excipient Profile	31
3.1	Memantine	31
3.1.1	Dosage and Administration	31
3.1.2	Mechanism of action of Memantine	31
3.1.3	Pharmacokinetics	32

3.2	PLGA.....	33
3.3	Pluronic F-127.....	34
3.4	Polyethylene Glycol (PEG)	35
3.5	Bone Marrow stem cell (BMSCs)	37
4	Aim and objective	41
4.1	Aim	41
4.2	Objectives	41
5	Materials and Methods	45
5.1	Preparation of nanoscaffolds	45
5.2	Methods for determination of response variables.....	45
5.2.1	Optimization of nanoscaffolds.....	45
5.2.2	Determination of Drug Loading (%)	46
5.3	Characterization of Optimised Memantine loaded Nanoscaffolds	46
5.3.1	Surface morphology (SEM).....	46
5.3.2	Entrapment Efficiency and Drug Loading.....	47
5.3.3	Biodegradability study.....	47
5.3.4	Preparation of Pegylated Memantine loaded self-assembled PLGA Nanoscaffolds (PEG-MEM-PLGA) SANs.....	47
5.3.5	Characterization of (PEG-MEM-PLGA) SANs	49
5.4	<i>In-vitro</i> studies	51
5.4.1	Drug Release study and Kinetic model	51
5.4.2	Blood-brain barrier permeation assay (PAMPA-BBB).....	52
5.5	<i>Ex-vivo</i> Study.....	53
5.5.1	Animal experiments.....	53
5.5.2	Enzyme Kinetic study against Acetylcholinesterase (AChE), Butyrylcholinesterase (BUCHE and β -Secretase in Cortex and Hippocampus	53
5.5.3	Hemocompatibility study	54
5.6	Stem cell grafting.....	55
5.6.1	Neurobehavioral studies	56
5.6.2	Scopolamine induced amnesia model and treatment groups.....	56
5.6.3	Y-Maze Test	56
5.6.4	Morris Water Maze Test.....	57
5.7	Estimation of pro-inflammatory cytokines.....	58
5.7.1	Animal model and treatment groups	58

5.8	Pharmacokinetic and Biodistribution study.....	60
5.8.1	Animal Experiments	60
5.8.2	High Performance Liquid Chromatography analysis of the pharmacokinetic and Biodistribution study	61
5.9	Pharmacodynamic study	62
5.10	Biochemical parameters.....	63
5.11	Histological analysis	63
5.12	Statistical analysis.....	63
6	Results and Discussion.....	67
6.1	Experimental design	67
6.1.1	Statistical analysis of experiments	70
6.1.2	Effect on Porosity (%) and Drug loading	70
6.1.3	Prediction of optimized formulation.....	71
6.1.4	Analysis of Variance.....	72
6.1.5	Response Surface methodology.....	73
6.2	Characterization of Memantine loaded nanoscaffolds.....	78
6.2.1	Surface morphology (SEM).....	78
6.2.2	Percentage porosity	80
6.2.3	Entrapment Efficiency and Drug Loading.....	80
6.2.4	Biodegradation study	81
6.2.5	Swelling index and water uptake study	81
6.3	Characterization of (PEG-MEM-PLGA) SANs	82
6.3.1	Drug and polymer incompatibility study (FTIR) and Thermal Characteristics (TGA and DSC)	82
6.3.2	Surface morphology (SEM, Particle size and Zeta potential)	84
6.3.3	Determination of porosity (%).....	87
6.3.4	Entrapment Efficiency	87
6.3.5	Biodegradation and Swelling index study	87
6.3.6	Stability Studies	89
6.3.7	<i>In-vitro</i> studies	89
6.3.8	<i>Ex-vivo</i> study.....	93
6.3.9	Hemocompatibility study.....	99
6.3.10	Neurobehavioral Effects	100
6.4	Pharmacokinetic and Biodistribution study.....	109
6.4.1	HPLC Study.....	109

6.5	Histological study	121
6.5.1	Histological studies.....	121
6.6	Estimation of pro-inflammatory cytokines.....	123
6.6.1	Expression of pro-inflammatory cytokines in serum, cerebral, hepatic and renal tissues.....	125
6.6.2	Involvement of cytokines in AD	128
6.6.3	Therapeutic effect of Memantine and nanoscaffolds on inflammation in AD	129
6.6.4	Statistical Analysis	134
7	Summary	137
8	Conclusion	147
9	References.....	153
10	Appendix.....	183
