

LIST OF FIGURES

CHAPTER-1

Figure 1.1: A summary of the design process for peptide therapeutics and their associated delivery systems.....3

Figure 1.2: Various nanocarriers for delivery of peptides12

CHAPTER-2

Figure 2.1: Various challenges of delivering macromolecules..... 22

Figure 2.2: A) Chemistry of vasopressin and B) site of its release from the pituitary gland.....48

Figure 2.3: Signalling mechanism of angiotensin in body 67

CHAPTER-3

Figure 3.1: HPLC chromatogram for oxytocin standard and samples.....144

Figure 3.2: Linearity plot of oxytocin..... 145

Figure 3.3: HPLC chromatogram for vasopressin standard..... 151

Figure 3.4: Linearity plot of vasopressin 152

Figure 3.5: Blank-diluent chromatogram of angiotensin-II standard 157

Figure 3.6: Blank-diluent chromatogram of angiotensin-II API 157

Figure 3.7: Linearity plot of angiotensin II..... 157

CHAPTER-4

Figure 4.1: Assay of Oxytocin in RTI formulation at different pH at Initial level and after 3M at 25 °C/40% RH. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit. 172

LIST OF FIGURES

Figure 4.2: Osmolarity of Oxytocin RTI formulation at different pH at Initial level and after 3M at 25 °C/40% RH. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.	172
Figure 4.3: Decision tree for sterilization choices for aqueous based products.....	175
Figure 4.4: Short term Stability Plot of Assay (mean) at different conditions and at different time point for optimized Oxytocin formulation (Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.	177
Figure 4.5: Short term stability plot of pH at different conditions and at different time point. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	177
Figure 4.6: Short term stability plot of osmolarity at different conditions and at different time point. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	179
Figure 4.7: Assay of Oxytocin in RTI formulation after different freeze (-20 °C) thaw (+40 °C) cycles. Graph presents mean of individual value. Red line in graph presents upper control limit (UCL) and lower control limit (LCL).....	180
Figure 4.8: Sensitivity to gas: Assay of Oxytocin in RTI formulation after purging with different gases. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.	182
Figure 4.9: Assay of Oxytocin at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control	189
Figure 4.10: pH of Oxytocin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	189

LIST OF FIGURES

Figure 4.11: Osmolarity of Oxytocin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	190
Figure 4.12: Assay of Oxytocin in final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.	191
Figure 4.13: pH of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	191
Figure 4.14: Osmolarity of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	193
Figure 4.15: Shelf Life Plot for All Batches	194
Figure 4.16: Shelf Life Plot for All Batches	195
Figure 4.17: Shelf Life Plot for Batch 0.02	195
Figure 4.18: Shelf Life Plot for Batch 0.08	196
Figure 4.19: Shelf Life Plot for All Batches	196
Figure 4.20: Shelf Life Plot for All Batches	197
Figure 4.21: Shelf Life Plot for All Batches	197
Figure 4.22: Assay of Vasopressin at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	209
Figure 4.23: pH of vasopressin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	210

LIST OF FIGURES

Figure 4.24: Osmolarity of vasopressin formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.	210
Figure 4.25: Assay of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	213
Figure 4.26: pH of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	213
Figure 4.27: Osmolality of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	214
Figure 4.28: Shelf Life Plot for Batch 1	215
Figure 4.29: Shelf Life Plot for Batch 2	215
Figure 4.30: Shelf Life Plot for Batch 1 & 2	216
Figure 4.31: Shelf Life Plot for Batch 1	216
Figure 4.32: Shelf Life Plot for Batch 2	217
Figure 4.33: Shelf Life Plot for Batch 1 & 2	217
Figure 4.34: Shelf Life Plot for All Batches	218
Figure 4.35: Shelf Life Plot for Batch 1	218
Figure 4.36: Shelf Life Plot Batch 2	219
Figure 4.37: Shelf Life Plot for All Batches.....	219
Figure 4.38: Shelf Life Plot for Batch 1	220
Figure 4.39: Shelf Life Plot for Batch 2	220

LIST OF FIGURES

Figure 4.40: Shelf Life Plot for All Batches	221
Figure 4.41: Shelf Life Plot for All Batch	221
Figure 4.42: Assay of Angiotensin-II at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	229
Figure 4.43: pH of Angiotensin-II formulation at different stability conditions and time points.....	230
Figure 4.44: Osmolarity of Angiotensin-II formulation at different stability conditions and time points: Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.	232
Figure 4.45: Assay of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	232
Figure 4.46: pH of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL (upper control limit) and lower control limit.....	233
Figure 4.47: Osmolality of final formulation at different stability conditions and time points. Graph presents mean of individual value. Red line in graph presents UCL and LCL.	233
Figure 4.48: Shelf Life Plot for Batch 1 & 2	236
Figure 4.49 : Shelf Life Plot for All Batches	236
Figure 4.50: Shelf Life Plot for All Batches	237
Figure 4.51: Shelf Life Plot for Batch 1	237
Figure 4.52: Shelf Life Plot for Batch 2	238

LIST OF FIGURES

Figure 4.53: Shelf Life Plot for Batch Shelf Life Plot for All Batches	238
Figure 4.54: Shelf Life Plot for Batch Shelf Life Plot for All Batches	239
Figure 4.55: Shelf Life Plot for Batch Shelf Life Plot for All Batches	239
Figure 4.56: Final all three Ready to use formulations.....	243
Figure 4.57: % Hemolysis for RTI formulation of Oxytocin, Vasopressin, Angiotensin-II	246

CHAPTER-5

Figure 5.1: Schematic presentation of fabrication of liposomes.....	256
Figure 5.2: Graph of particle size analysis (A), and HR-TEM image (B) of oxytocin loaded liposomes and vasopressin loaded liposomes respectively.....	273
Figure 5.3: % Cell viability after the exposure of different formulations (plain and drug-loaded) after 24 h, 48 h & 72 h incubation	275
Figure 5.4: Morphological observation of SH-SY5Y cells treated with High dose of vasopressin, oxytocin, vasopressin loaded liposomes and oxytocin loaded liposomes for 48 h at 20× magnification.	276
Figure 5.5: Cell uptake of SH-SY5Y cells treated with High dose of vasopressin, oxytocin, vasopressin loaded liposomes and oxytocin loaded liposomes for 48 h at 20× magnification.	277
Figure 5.6: ROS generation of SH-SY5Y cells treated with control, Oxytocin, vasopressin, OXT-Liposomes and Vaso-liposomes at 100µg/mL for 48 h at 20× magnification.	278
Figure 5.7: Acridine orange/Ethidium bromide dual staining studies of control, Vasopressin, vasopressin loaded liposomes, oxytocin and oxytocin loaded liposomes at high dose in SH-SY5Y cells for 48 h at 20× magnification.	280
Figure 5.8: Determination of transcriptional gene expression of inflammatory, signalling and apoptotic markers	281

LIST OF FIGURES

Figure 5.9 [A] & [B]: Expression of FOXO-1 gene following the LPS after various formulations treatment	283
Figure 5.10: Pharmacological evaluation of formulations in Balb/C mice by Morris maze water studies. free oxytocin solution and oxytocin encapsulated liposomes administered via i.n. & i.v. route. Data are represented for control (Ctrl), scopolamine treated (SCP), oxytocin loaded liposomes intranasal (i.n) and intravenous (i.v.), oxytocin (i.n.) and oxytocin (i.v.). Figure A for acquisition trials, while figure B represents the time in seconds required by animal to reach the hidden platform.	285
Figure 5.11: Gene expression of brain with different treatment groups. Data are represented for control (Ctrl), scopolamine treated (SCP), oxytocin loaded liposomes intranasal (IN) and intravenous (IV), oxytocin (IN) and oxytocin (IV).....	288
Figure 5.12: Biochemical estimation of brain homogenates with different treatment groups. Data are represented for control (Ctrl), scopolamine treated (SCP), oxytocin loaded liposomes intranasal (IN) and intravenous (IV), oxytocin (IN) and oxytocin (IV)	289
Figure 5.13: Images of histopathology evaluation of brain Hippocampus of Balb/C with scale 50 µm. Animals treated with free oxytocin solution and oxytocin encapsulated liposomes administered via i.n. & i.v. route. Data are represented for control (liposomes placebo), scopolamine treated (SCP), oxytocin loaded liposomes intranasal (i.n) and intravenous (i.v.), oxytocin (i.n.) and oxytocin (i.v.).....	291
Figure 5.14: Pharmacological evaluation of formulations in Balb/C mice by Morris maze water studies. free vasopressin solution and vasopressin encapsulated liposomes administered via i.n. & i.v. route. Data are represented for control (Ctrl), scopolamine treated (SCP), vasopressin loaded liposomes intranasal (i.n) and intravenous (i.v.), vasopressin (i.n.) and vasopressin (i.v.). Figure A for acquisition trials, while figure B represents the time spent by animal to reach the hidden platform in.....	293
Figure 5.15: Gene expression level of brain samples. Data are represented for control (Ctrl), scopolamine treated (SCP), Vasopressin-loaded Liposomes intranasal (i.n.) and intravenous (i.v.), vasopressin (i.n.) and vasopressin (i.v.)	294

LIST OF FIGURES

Figure 5.16: Biochemical estimation of brain homogenates. Data are represented for control (Ctrl), scopolamine treated (SCP), vasopressin loaded liposomes intranasal (i.n) and intravenous (i.v.), vasopressin (i.n.) and vasopressin (i.v.).....	295
Figure 5.17: Images of histopathology evaluation of brain Hippocampus of Balb/C with scale 50 µm. Animals treated with free vasopressin solution and vasopressin encapsulated liposomes administered via i.n. & i.v. route. Data are represented for control (Naïve), scopolamine treated (SCP), SCP+vasopressin loaded liposomes intranasal (i.n) and intravenous (i.v.), SCP+ vasopressin (i.n.), and SCP+ Vasopressin (i.v.).scopolamine treated (SCP), SCP+vasopressin loaded liposomes intranasal (i.n) and intravenous (i.v.), SCP+ vasopressin (i.n.), and SCP + Vasopressin (i.v.)	296

CHAPTER-6

Figure 6.1: Structure of prepared AEEA-oxytocin conjugate	303
Figure 6.2: Schematic presentation of synthesis of NH ₂ -AEEA-OXYTOCIN	304
Figure 6.3: FT-IR Spectra of AEEA-Oxytocin.....	315
Figure 6.4: NMR spectra of oxytocin & AEEA-Oxytocin	316
Figure 6.5: Mass spectra of Oxytocin and direct mass spectra of AEEA-oxytocin	317
Figure 6.6: % Cell viability after the exposure of oxytocin and AEEA-oxytocin after 24 h, 48 h & 72 h incubation.....	319
Figure 6.7: % of Cell uptake after the exposure of oxytocin and AEEA-oxytocin after 1 h, 2 h & 3 h incubation (n=3).....	320
Figure 6.8: Morphological observation of SH-SY5Y cells treated with high dose (100 µg/mL) of oxytocin and AEEA-Oxytocin for 48 h at 20× magnification	321
Figure 6.9: Cell uptake of SH-SY5Y cells control, treated with High dose (100 µg/mL) of oxytocin and AEEA-oxytocin for 48 h at 20× magnification.....	322

LIST OF FIGURES

Figure 6.10: ROS generation of SH-SY5Y cells treated with control, oxytocin and AEEA-oxytocin at 100 µg/mL for 48 h at 20× magnification.....	323
Figure 6.11: Acridine orange/Ethidium bromide dual staining studies of control, oxytocin and AEEA-oxytocin at high dose in SH-SY5Y cells for 48 h at 20× magnification.....	324
Figure 6.12: Determination of transcriptional gene expression of inflammatory, signalling and apoptotic markers	326
Figure 6.13: Expression of FOXO-1 gene following the LPS after various formulations treatment	327
Figure 6.14: Pharmacological evaluation of formulations in Balb/C mice by MWM studies: free oxytocin solution and AEEA-oxytocin solution administered via i.n. route. Data are represented for control (Ctrl), scopolamine treated (SCP), SCP + oxytocin (i.n) and SCP + AEEA-oxytocin (i.n). Figure A for acquisition trials, while figure B represents time required in seconds by mice to reach the hidden platform	328
Figure 6.15: Gene expression of isolated brain. Data are represented for control (Ctrl), scopolamine treated (SCP), SCP + oxytocin (i.n) and SCP + AEEA-oxytocin (i.n).....	332
Figure 6.16: Biochemical estimation of homogenated brain. Data are represented for control (Ctrl), scopolamine treated (SCP), SCP + oxytocin (i.n) and SCP + AEEA-oxytocin (i.n).....	334
Figure 6.17: Images of histopathology evaluation of brain Hippocampus of Balb/C with scale 50 µm. Animals treated with free oxytocin and AEE-Oxytocin solution via i.n. route. Data are represented for control, scopolamine treated (SCP), SCP+OXT intranasal (i.n), SCP+AEEA-OXT (i.n.)	335