Chapter 15

Summary and Conclusions

SUMMARY AND CONCLUSIONS

Schizophrenia is a severe neuropsychiatric illness that usually begins during adolescence or early adulthood, and affects people of all age groups from either developing or industrialized nations. One of the major hurdle towards effective treatment of Schizophrenia is patient non-compliance. Failure to adhere to the dosage regimen can led to relapse which can be more deadly than the first psychotic episode. The currently available conventional dosage forms are mostly immediate release, require frequent dosing and are associated with non-compliance. Recent findings reveal that some commonly used atypical antipsychotics are actively effluxed out of the brain by the transmembrane energy dependent protein: P-Glycoprotein, thus failing to reach the brain in therapeutic concentrations. Thus, effective treatment of schizophrenia requires development of novel drug delivery systems which can sustain blood drug concentrations for an extended period of time, avoiding frequent dosing. Development of some drug delivery systems that can inhibit the efflux mechanism present at the blood brain barrier can help the drug reach the brain in therapeutic concentrations. Recent research across the globe has been focused on the potential use of biodegradable polymer (like poly lactide co-glycolide 50:50) based microsphere depot formulation for extending the drug release in vivo from a period of 7 days to 3 months. Several other drug delivery systems like nanoparticles, nanosuspensions, liposomes, microemulsions have been tried to enhance the brain delivery of centrally acting drugs by bypassing the blood brain barrier.

This thesis work explains the development, characterization and in vivo evaluation of Poly lactide co-glycolide 50:50 (PLGA 50:50) based microsphere depot formulation meant for intramuscular injection for combating the patient non-compliance. Development, characterization and in vivo evaluation of drug nanosuspensions and solid lipid nanoparticles for effective delivery of the drug to brain are also investigated.

Chapter 1 contains introduction, present chemotherapy available for Schizophrenia and objective behind the present study. Chapter 2 examines the literature related to Schizophrenia, blood brain barrier and its properties, P-Glycoprotein and its functions, polymers used for the preparation of the drug delivery system. Chapter 3 contains the profiles of the drugs used for the present study.

Chapter 4 explains the analytical method development for the estimation of the Ziprasidone and Olanzapine as the bulk drug, in the formulation and in rat plasma. Non – interference of the polymers (Poly lactide co-glycolide in case of Ziprasidone; Glyceryl mono stearate, Glyceryl distearate, Glyceryl tristearate in case of Olanzapine) and surfactants (Polyvinyl alcohol in case of Ziprasidone; Poloxamer 188, Poloxamer 407, Vitamin E TPGS, Gelucire® 44/14 and Transcutol® in case of Olanzapine) used in the λ_{max} of the drug was also confirmed. The analytical methods were then evaluated for accuracy and precision. The methods were also subjected to statistical analysis and the various statistical parameters were established. Results revealed that the analytical methods were accurate and highly precise. A simple spectrophotometric method was developed for the estimation of the water soluble dye Rose Bengal in distilled water. Rose Bengal partition studies were performed to study the nanoparticle surface hydrophobicity. For determination of Ziprasidone in rat plasma and different tissue homogenates, a sensitive, accurate and precise fluorimetric estimation method was developed. Ziprasidone was extracted from the plasma using methyl - *t* -butyl ether. The developed method gave high relative fluorescence intensity values of Ziprasidone in plasma and all the tissue homogenates with good correlation coefficient values. This study reveals the high potential of the fluorimetric estimation of Ziprasidone in biological samples such as plasma and the tissue homogenates of rat.

Chapter 5 reviews the different radiolabeling techniques available and explains the radiolabeling studies involving the labeling of Olanzapine solution, Olanzapine nanosuspension and the different Olanzapine loaded solid lipid nanoparticles. Olanzapine and its different nanoparticle formulations were radiolabelled with ^{99m}Tc by reduction using stannous chloride. The different parameters like the amount of stannous chloride required, pH of the medium and incubation time was studied and optimized. The invitro stability of the different radiolabelled formulations was determined by DTPA and Cysteine challenge transchelation test. The low degree of transchelation revealed the high binding efficiency of ^{99m}Tc and hence high invitro stability. Serum stability studies upto a period of 24 hours revealed that the radiolabelled complexes were stable and indicated their potential use as biomarkers for biodistribution and gamma scintigraphy studies.

Chapter 6 explains the formulation development, characterization and stability testing of Ziprasidone loaded PLGA 50:50 microspheres. ZB loaded PLGA 50:50 MS were prepared by simple o/w emulsification solvent evaporation technique. The suitable organic (oil) phase was optimized by initial partition coefficient studies. The ideal particle size for an intramuscular depot formulation is 25-180μm and preferable around 50μm. Hence, a complete study on the effect of stabilizer concentration and stirring speed on the particle size were optimized and the mean particle size was maintained between the suggested range suitable for intramuscular depot. The effect of drug to polymer ratio was studied and the maximum obtainable entrapment efficiency was achieved. The invitro release was estimated by using a rotating bottle apparatus for a period of 35 days. The in vitro release kinetics was found to follow Higuchi's release pattern. The apparent diffusion coefficient values were also calculated. Particle morphology was studied by light microscopy. Surface morphology of the initial micropsheres and microspheres after 35 days dissolution was studied by both scanning electron microscopy and light microscopy. Results revealed that the microspheres were spherical and had a smooth surface texture. The study of surface morphology after 35 days dissolution revealed that the microspheres were fragmented indicating that the release mechanism is primarily by surface erosion. DSC and XRD were performed to study the crystallinity status of ZB after incorporation into microspheres. Stability studies of the lyophilized micropsheres revealed that the microspheres were stable at both 4° and ambient room temperature (27-30°C) for a period of 6 months. These results indicate the possible application of these biodegradable microspheres as an intramuscular depot for sustained release of Ziprasidone in vivo.

Chapter 7 describes the pharmacokinetics of Ziprasidone loaded PLGA 50:50 microspheres prepared by administration through intramuscular route into rats. The plasma concentration versus time profiles were plotted for the plain ZB solution and ZB loaded PLGA 50:50 MS dispersion. In vivo, the PLGA microsphere formulation gave ZB blood level till a period of 17 days in case of 4mg depot and 10 days in case of 2mg depot indicating the sustained release potentiality of the formulated PLGA microspheres. Steady state plasma ZB levels between 57ng/mL to 67ng/mL were obtained when between day 2 and day 10 post injection for 4mg depot and 38ng/mL to 53ng/mL were obtained when between day 2 and day 10 post injection for 2 mg depot. Interpreting the experimental results, it appears that the above formulated

microsphere formulation can be a potential long acting formulation for combating non-compliance associated with Schizophrenia.

Chapter 8 explains the formulation of Ziprasidone nanosuspension prepared by pearl milling technique using different sized zirconium beads. A complete study of the effect of the different sized beads, volume of beads used for milling, surfactant concentration and milling time on the final mean particle size of the nanosuspension is described. Pearl milling of ZB for 8 hours in the presence of poloxamer 407 as surfactant using zirconium oxide beads (75:25 ratio of size 0.4- 0.7mm: 1.2-1.4mm) yielded nanoparticles of smallest size (309 ± 4.28nm). The nanosuspension dispersions were successfully converted into the powder form by either lyophilization (ZBLNS) or by spray drying (ZBSNS) to increase the shelf life. Transmission electron microscopy studies revealed that the drug nanoparticles were spherical in shape and the size distribution was mono-disperse with low polydispersity index. DSC and XRD studies revealed that there was a change in the crystallinity of Ziprasidone after conversion into nanosuspension state. Electrolyte flocculation test revealed that the nanosuspension formulation tended to agglomerate in electrolyte concentrations beyond 0.3M sodium sulphate indicating instability of the nanosuspension beyond this electrolyte cocncentration. The nanosuspension forms of ziprasidone improved its solubility and dissolution in vitro. Stability studies of the nanosuspensions in the dispersion form revealed particle size growth and zeta potential change revealing the instability of the nanosuspensions in the dispersion state. Stability studies were also conducted for ZBLNS and ZBSNS in the powder form for a period of 6 months. The formulations were found to be stable throughout the period studied in terms of particle size, reconstitution time and invitro release at 2-8°C, ambient temperature (27-30°C) and 40°C. These encouraging in vitro results revealed the need for invivo blood clearance and brain deposition studies to exploit these nanosuspensions as alternative parenteral dosage forms to the existing marketed ziprasidone injection.

Chapter 9 describes the pharmacokinetics and biodistribution studies of Ziprasidone nanosuspensions prepared by pearl milling and were administrated through the intramuscular route into rats. Formulation of Ziprasidone into nanosuspension form greatly altered its residence time in the blood and also its biodistribution. In vivo, the nanosuspensions exhibited higher blood circulation time and brain concentration compared to the ziprasidone solution,

indicating the potentiality of nanosuspension forms. About 2.51 fold and 2.22 fold increase in $t_{1/2}$ ($p<0.005$) respectively ZBLNS and ZBSNS was observed incomparison to the plain ZB solution. These can be attributed to the steric stabilization imparted on the drug nanoparticle by the surface adsorbed poloxamer 407. However, there was insignificant difference in the residence time in blood of ZBLNS and ZBSNS. Biodistribution studies showed increased brain concentrations of ZB in the brain after i.m. administration of the nanosuspension than from the plain ZB solution at 1 hour, 4 hour and 8 hour post injection. ZB concentration in the RES organs like liver, lung, spleen and kidney was also significantly reduced ($p<0.001$) when administered in the nanosuspension form. These data substantiate the potential of the above formulated poloxamer coated ZB nanosuspension as a long circulating system in blood and higher brain deposition. Looking at the experimental results, it appears that the proposed nanosuspension forms can be an alternative to the commercially available ziprasidone parenteral formulation for intramuscular injection.

Chapter 10 describes the methodology and characterization techniques used in the formulation development of Olanzapine nanosuspension. Poloxamer 407 stabilzed (POL NS) and TPGS stabilized (TOL NS) Olanzapine nanosuspension were formulated by pearl milling technique using zirconium beads of different sizes. A complete study of the effect of the different sized beads, volume of beads used for milling, surfactant concentration and milling time on the final mean particle size of the nanosuspension is described. The nanosuspension dispersions were successfully converted into the powder form by lyophilization to increase the shelf life. Transmission electron microscopy studies revealed that both the drug nanoparticles were spherical in shape and the size distribution was mono-disperse with low polydispersity index. DSC and XRD studies revealed that there was a change in the crystallinity of olanzapine after conversion into nanosuspension state. Electrolyte flocculation test revealed that the nanosuspension formulations tend to agglomerate in electrolyte concentrations beyond 0.6M sodium sulphate incase of POL NS beyond 0.3M sodium sulphate incase of TOL NS indicating the higher resistance to invitro electrolyte induced flocculation of the POL NS. The nanosuspension forms of Olanzapine improved its solubility and dissolution in vitro. Stability studies were also conducted for POL NS and TOL NS in the powder form for a period of 6 months. The formulations were found to be stable throughout the period studied in terms of particle size, reconstitution time and invitro release at 2-8°C, ambient temperature (27-30°C)

and 40°C. Preliminary studies gave encouraging results for possible application of these nanosuspensions as long circulating drug carriers in blood and higher brain delivery on comparison to the plain olanzapine solution.

Chapter 11 describes the pharmacokinetics and biodistribution studies of Olanzapine nanosuspensions prepared by pearl milling administrated through the intravenous route into rabbits. Blood clearance and biodistribution studies were carried using radiolabeling technique. Both the nanosuspension forms revealed higher residence times in the blood when compared to the plain drug solution. These can be attributed to the steric stabilization imparted on the drug nanoparticle by the surface adsorbed poloxamer 407 or TPGS. However, there was insignificant difference in the residence time in blood of POL NS and TOL NS. These data indicate the long circulation capability of the formulated nanosuspension formulations. Marked change in the biodistribution pattern was observed after administration of Olanzapine in the nanosuspension form. Plain OL solution exhibited higher concentrations in the organs of the reticuloendothelial system (RES), such as liver, spleen, and lung when compared to the nanosuspension formulations. The overall uptake of POL NS and TOL NS by liver, lung, spleen and kidney was significantly lower than the free OL ($p < 0.005$). The concentration of TOL NS was relatively higher in brain (9-fold and 2.42-fold higher than plain OL solution and POL NS at 1 hour respectively). Reduced hydrolyzed technicium tends to accumulate in the stomach and intestine. A very low radioactivity was recovered from stomach and intestine and showed constancy with time indicating the in vivo stability of the radiolabeled complexes. The images clearly showed the difference in the concentration of the radiolabelled complex in the brain after intravenous injection of POL BS and TOL NS. These data substantiate the potential of the above formulated poloxamer 407 or TPGS stabilized OL nanosuspension as a long circulating system in blood and higher brain deposition.

Chapter 12 describes the effect of the lipid matrix on the physical stability, entrapment efficiency and invitro release of Olanzapine from SLN dispersions from hot melt high pressure homogenization technique. For this purpose, lipids of different chemistry were selected namely glyceryl mono stearate (GMS) Precirol ATO 5 (PRE), glyceryl tristearate (GTS) and Witepsol E85 (WE 85). The effect of homogenization pressure and homogenization cycle number on the size of nanoparticles were determined and optimized. The effect of surfactant concentration on

the mean particle size diameter was also determined. Crystallinity changes in the lipid after incorporation of Olanzapine was studied by DSC and XRD. Electrolyte induced flocculation test was performed to study the in vitro steric stability of the nanoparticles. All the four SLN dispersions showed signs of flocculation when the concentration of sodium sulphate was increased above 0.6M. Beyond this concentration, a sharp increase in the flocculation was observed indicating instability beyond this electrolyte concentration. Surface hydrophobicity was determined by Rose Bengal adsorption method and was in the order of OL loaded GTS SLN > OL loaded PRE SLN > OL loaded WE 85 SLN > OL loaded GMS SLN. The nanoparticles were subjected for short term stability studies for a period of 4 months at 4°C and 40°C and the optimum stability conditions were determined. The nanoparticle dispersions were found to be unstable during the course of the stability study showing increase in particle size and change in zeta potential. Invitro release of Olanzapine from the different SLN dispersions was studied in 0.1N HCl and phosphate buffer pH 7.4. The OL loaded GTS SLN showed the highest sustained release among the four SLN dispersions in both the release media studied.

Chapter 13 describes the effect of the stabilizer on the physical stability of the different Olanzapine loaded SLNs from hot melt high pressure homogenization technique. It was found that SLN dispersions prepared from glyceryl tristearate gave the highest entrapment among the different lipids used (Chapter 12). In this chapter, an attempt has been made to explore the effect of surfactant type in the nanoparticle stabilization, crystallinity changes and physical stability of the SLN dispersions. For this, three surfactants were selected namely Gelucire® 44/14 (PEG-32 glyceryl laurate), Transcutol® (diethylene glycol monoethyl ether), and Poloxamer 188 (co-block polymer of polyoxypropylene and polyoxyethylene). Gelucire® 44/14 and Transcutol® are proven P-glycoprotein inhibitors and can effectively increase the brain deposition in vivo by inhibiting the P-glycoprotein present at the blood brain barrier. The effect of homogenization pressure and homogenization cycle number on the size of nanoparticles were determined and optimized. The effect of different surfactant concentration on the mean particle size diameter was also determined. SLN dispersions prepared with Transcutol® alone lead to dispersions with high polydispersity index. Hence Poloxamer 188 was added to avert this problem. Crystallinity changes in the lipid after incorporation of Olanzapine was studied by DSC and XRD. Electrolyte induced flocculation test was performed

to study the in vitro steric stability of the nanoparticles. All the three SLN dispersions showed signs of flocculation when the concentration of sodium sulphate was increased above 0.6M. Beyond this concentration, a sharp increase in the flocculation was observed. Surface hydrophobicity was determined by Rose Bengal adsorption method and was in the order of Gelucire® 44/14 stabilized SLN > Transcutol® stabilized SLN > Poloxamer 188 stabilized SLN. The surface hydrophobicity was in correlation with the HLB values of the surfactant used for stabilization. Poloxamer 188 being the most hydrophilic of the surfactants studied gave the least surface hydrophobicity. The nanoparticles were subjected for short term stability studies for a period of 4 months at 4°C and 40°C and the optimum stability conditions were determined. The nanoparticle dispersions were found to be unstable during the course of the stability study showing increase in particle size and change in zeta potential. Invitro release of Olanzapine from the different SLN dispersions was studied in 0.1N HCl and phosphate buffer pH 7.4. There was no significant change in the invitro release between the different OL loaded SLNs. The above invitro release data caused interest in further studies in rats for studying the in vivo circulation times.

Chapter 14 describes the pharmacokinetics and biodistribution studies of Olanzapine loaded SLN prepared by hot melt high pressure homogenization technique after intravenous administration into rabbits. Blood clearance and biodistribution studies were carried using radiolabeling technique. All the SLN dispersions exhibited higher residence times in the blood when compared to the plain drug solution. These can be attributed to the steric stabilization imparted on the drug nanoparticle by the surface adsorbed poloxamer 188, Gelucire® 44/14 and Transcutol®. However, there was insignificant difference in the residence time in blood between the different SLN dispersions. These data indicate the long circulation capability of the formulated solid lipid nanoparticle formulations. Such differential blood kinetics would also result in different biodistribution patterns. Marked change in the biodistribution pattern was observed after administration of Olanzapine encapsulated in the SLN form. OL deposition after administration as OL in Gelucire® 44/14 solution (GOL solution) and was OL in Transcutol® solution (TROL solution) studied as a negative Control to investigate the effect of the Pgp inhibitor alone in helping the drug to cross the BBB. Poloxamer 188 stabilized as the positive control to study the effect of nanoparticle alone in helping the drug to cross the BBB as Poloxamer does not inhibit P-glycoprotein. Plain OL solution exhibited higher

concentrations in the organs of the reticuloendothelial system (RES), such as liver, spleen, and lung when compared to the SLN formulations. The overall uptake of the OL loaded SLN by liver, lung, spleen and kidney was significantly lower than the free OL ($p < 0.005$). Insignificant radioactivity was recovered from stomach and intestine and showed constancy with time indicating the in vivo stability of the radiolabelled complexes as reduced hydrozed technicium tends to accumulate in these organs.

Among the different SLN formulations, the concentration of GOL SLN was relatively higher in brain (7.75 fold, 6.71 fold and 3.20 fold higher than plain OL solution at 1 hour, 4 hour and 8 hour respectively). When the brain concentrations of GOL SLN and GOL solution were compared, 2.38 fold increase in the brain was achieved with the GOL SLN formulation. When the brain concentrations of GOL SLN and POL188 SLN were compared, 3.26 fold increase in the brain was achieved with the GOL SLN formulation. These results denote that nanoparticles stabilized with Pgp inhibitors are a better option for targeting the drug to the brain when compared to the plain drug - surfactant solution and nanoparticle with a surfactant which is not a Pgp inhibitor. Similar results were achieved TROL SLNs. The SLN stabilized with Transcutol® gave higher brain concentrations when compared to TROL solution. However, there was no significant difference in the brain uptake of OL between the GOL solution, TROL solution and POL188 SLN. Gamma scintigraphic images clearly showed the difference in the concentration of the radiolabelled complex in the brain after 1 hour post intravenous injection of GOL SLN and TROL SLN. These data substantiate the potential of the above formulated Gelucire® 44/14 or Transcutol® stabilized olanzapine nanoparticles as a long circulating system in blood and higher brain deposition.

Presently all antipsychotics are administered through the intramuscular route when given parenterally. We wanted to investigate the change in blood clearance patterns after intravenous and intramuscular administration. The peak plasma radioactivity was around 30 minutes for the OL solution and the different OL loaded SLN dispersions when administered through the intramuscular route. The intravenous route of administration revealed significantly higher radioactivity in the blood when compared to the intramuscular route ($p < 0.005$). Distribution pattern to the RES organs also differed significantly ($p < 0.005$). There was a marked reduction in the liver uptake of the radiolabelled complexes by the liver after i.m. administration when

compared to the i.v. route. Uptake by the other organs did not differ significantly after i.m. administration. The intravenous route of administration was found to be more suitable since it yielded higher brain deposition when compared to the intramuscular route of administration.

The present study demonstrates some new findings, which may be exploited in improving the therapeutic efficacy of antipsychotic agents using PLGA microsphere based depot/ nanosuspension/ lipid nanoparticle systems.

The results of this thesis work reveal that

1. The lyophilized Ziprasidone loaded PLGA microspheres were stable upto a period of 6 months at both 2-8°C and ambient room temperature (27-30°C)
2. PLGA polymer microsphere based intramuscular depot can sustain the plasma Ziprasidone levels for a period of two weeks.
3. Poloxamer 407 stabilized Ziprasidone nanosuspension can be prepared by pearl milling technique using zirconium beads with insignificant milling media residues and were stable in the powder form for a period of 6 months at both 2-8°C and ambient room temperature (27-30°C).
4. Nanosuspension form of Ziprasidone exhibited higher blood residence times and brain deposition when compared to the plain drug.
5. Poloxamer 407 / TPGS stabilized Olanzapine nanosuspension can be prepared by pearl milling technique using zirconium beads with insignificant milling media residues and were stable for a period of 6 months in the powder form at 2-8°C and ambient room temperature (27-30°C).
6. Stable Olanzapine loaded solid lipid nanoparticles can be prepared by hot melt high pressure homogenization technique.
7. Olanzapine and the various Olanzapine nanoparticle formulations can be radiolabelled with ^{99m}Technicium by reduction using Stannous Chloride
8. Olanzapine nanosuspension and Olanzapine loaded Solid lipid nanoparticles stabilized with P-glycoprotein inhibitors exhibited higher residence times in blood compared to plain Olanzapine

9. Olanzapine nanosuspension and Olanzapine loaded Solid lipid nanoparticles stabilized with P-glycoprotein inhibitors revealed higher brain concentrations of the P-glycoprotein substrate.

However, extensive clinical trials need to be performed to establish the efficacy and safety of these novel drug delivery systems. We believe that these systems have the potential to improve the therapy of certain psychotic disorders. Their effective use will not only increase patient non compliance, it will possibly reduce the dose of the antipsychotic agent for a given degree of therapeutic response. Hence, these novel drug delivery systems can play an imperative role in annihilating the present shortcomings associated with the treatment of Schizophrenia and lead to efficient chemotherapy.