Chapter 11

Summary and Conclusion

Management of Dyslexia and ADHD

❖ Summary and Conclusion for Oral SMEDDS

11.1 Summary

Aim of the present work was to formulate Self-micro emulsifying drug delivery-based drug delivery system to improve the oral bioavailability of BCS class- II drug i.e. Modafinil and characterize for *in vitro, ex vivo* and *in vivo* potential for the management of ADHD and Dyslexia. Modafinil exhibit high permeability through biological membranes, but its absorption after oral administration is limited due to its low aqueous solubility. The influence of the oil, surfactant and co-surfactant on drug solubility and their proportion on the forming competent and stable drug loaded SMEDDS were examined.

11.1.1 Analytical Method

Interestingly, Modafinil did not produce any characteristic peak when scanned in the ultraviolet range between 200 and 400 nm. Therefore, mathematical calculation can be used for conversion of these peaks to first order derivative for identical analytical method. Both first order derivative UV spectroscopy and HPLC methods were validated for linearity, accuracy and precision. The validation parameters for UV spectroscopy method were found to meet the "readily pass criteria" and % RSD were found less than 2%. The % recovery was found to be between 98-102%, indicates that the method was accurate to estimate Modafinil in the range of 5-20 µg/ml. % RSD for intraday and interday of UV spectroscopy method for Modafinil at 4 different concentration levels and %RSD values obtained were less than 2.0% suggest that these methods have good precision and reproducibility. LOD and LOQ were found to be 0.0392 µg/ml and 0.1188 µg/ml respectively. Initially, calibration curve of Modafinil was prepared by UV spectroscopy to estimate the drug concentration in formulation but interference with the excipients were found, therefore UV spectroscopy method was not further used for estimation of the Modafinil. To overcome this problem; solubility determination of Modafinil in various oils, surfactant and co-surfactant, in dosage form and in diffusion medium was analyzed by developed and validated RP-HPLC analytical method. The elution was done with the flow rate of 1.0 ml/min of Methanol: Water : Acetic Acid (500 : 500 : 1) and the retention time (Rt) of Modafinil was found to be 8.56 ± 0.08 min at λmax of 220nm of detection. The linearity of the method was found to be in the range of 2.5-20µg/ml. The % recovery in between 98-102% showed that the method was accurate

to estimate Modafinil in 2.5-20 µg/ml range. The repeatability of the measurement was expressed in terms of % RSD and it was found to be less than 2% RSD for intraday and interday of Modafinil at different concentration levels, hence it was found that the analytical method were within the acceptable range indicating that these methods have excellent repeatability and intermediate precision. LOD and LOQ were found to be 0.043839 µg/ml and LOQ: 0.132847µg/ml respectively. The HPLC method for Modafinil was further analysed for interference with excipients and chromatograms showed no interference of excipients in the estimation of Modafinil. This validated HPLC analytical method was applied for analysis of Modafinil in plasma and aslo found linear in range of 1-20 µg/ml. Moreover, DSC was performed to characterize drug; and drug interaction with excipients was also confirmed by FT-IR analysis.

11.1.2. Formulation and Optimization of Modafinil SMEDDS

Various oils, surfactants and co-surfactants were screened as components of Modafinil SMEDDS based on the solubility and emulsifying ability each excipients. The ratio of various components was obtained by construction of ternary phase diagram. For the optimization of the formulation, D-optimal mixture design was applied using Design Expert software version 9.0. The optimized SMEDDS formulation consisted of 8% Oil (Clove oil), 65% Surfactant (Tween-80) and 27% Co-surfactant (Polyethylene glycol-400), Km value 1:2. This formulation was capable of giving microemulsion with globule size of 19.21±0.47nm and %Transmittance 99.515 ± 0.19 % for SMEDDS formulation on 1:100 times dilution.

11.1.3 Characterization of Modafinil SMEDDS

Optimized Modafinil SMEDDS was characterized for Thermodynamic stability testing, dispersibility, self-emulsification time, robustness on dilution (effect of pH and dilution factor), dye solubility, globule size, zeta potential, effect of pH, viscosity, % transmittance, conductance, viscosity, cloud point, assay and TEM analysis. The SMEDDS was found to be thermodynamically stable, easily dispersible, self-emulsification time 24±1.5 sec, robustness on the dilution, dye solubility test observed was devoid of any clump formation, globule size was found to be 19.21 ± 0.47nm with PDI 0.186 ± 0.27, zeta potential -2.73±0.14 mV shows a good stability with pH 6.28 ± 0.13, conductance 56.21 ± 0.37 µS/cm and Viscosity 1229.33 ± 0.57 cP. Assay 99.69 ± 0.23% shows stability of drug in formulation. Cloud point for SMEDDS was at the 76 ± 3°C.

11.1.4 Drug Release Study

11.1.4.1 *In vitro* drug release profile

In vitro drug release profile of Modafinil SMEDDS was performed by both the conventional dissolution methods as well as recently adopted diffusion sac method. In the conventional dissolution method; dissolution medium, 0.1 N HCl containing 0.5% SLS at 37 ± 0.50°C with rotating speed of 50 rpm, the drug released from SMEDDS was more than 80% in less than 10 min, which is approximately 7 times higher compared to drug. After 15 min, SMEDDS shows almost complete drug release within 30 min whereas only 50% drug release from pure drug after 4 hours. Furthermore, the dissolution profile of Modafinil from SMEDDS formulation was unaffected by the pH of the dissolution medium, with complete release achieved within 30 minutes in the both dissolution medium, 0.1 N HCl containing 0.5% SLS and phosphate buffer pH 6.8 containing 0.5% SLS at 37 ± 0.50°C with rotating speed of 50 rpm.

In vitro drug release profile through dialysis sac revealed that modafinil diffused from suspension was very less as compared to SMEDDS and shows that release of Modafinil from SMEDDS after 8 hr was 1.5 times higher as compared to drug suspension. It may be due to small globule size of SMEDDS formulation, provides an additional support for faster solubilization and diffusion from dialysis sac that result in faster release of drug than suspension. This study was continued for 24 hr and results showed the diffusion profile of modafinil from dialysis sac was improved for SMEDDS, because solubilized drug in SMEDDS easily permeable through dialysis sac and thus improved dissolution profile of modafinil.

11.1.4.2 *Ex vivo* drug release profile

Ex vivo drug release study was performed for the prediction of oral absorption from SMEDDS formulation. The study was carried out with the reconstituted SMEDDS filled in stomach and the intestinal lumen of scarified Male Sprague Dawley rats. It was observed that for the Stomach, Modafinil release/diffuse from the SMEDDS approximately 2.5 times higher than the drug suspension.

The release of the drug was found to be faster from the intestine in comparison to the stomach. After 8 hr, SMEDDS releases more than 90% of drug from the intestine, which was 1.7 times

higher compared to the drug suspension. Thus, the absorption of the drug from the intestine can be enhanced with SMEDDS formulations.

11.1.5 *In vivo* Pharmacokinetic Study

In vivo study was performed to compare pharmacokinetic data of optimized SMEDDS formulation with drug suspension and marketed product. All the formulations were orally administered to male Sprague-Dawley rats. The plasma concentration vs time curve in rats after a single oral dose of Modafinil loaded SMEDDS with compared to drug suspension, the Modafinil concentrations in rat plasma treated with SMEDDS were significantly higher ($p<0.05$) than those treated with suspension and marketed formulation at all the time points. Also the Cmax, Tmax, AUC, $t_{1/2}$ and MRT of Modafinil for Modafinil-loaded SMEDDS were significantly higher than that of Modafinil drug suspension and marketed formulation. The higher values of pharmacokinetic parameters observed in SMEDDS formulation shows improvement in rate and extent of drug concentration in the systemic circulation and finally results in enhancement in the bioavailability.

11.1.6 Stability Studies

The stability studies of Modafinil were carried out as per ICH guidelines. Physicochemical stability of Modafinil loaded SMEDDS was assessed under different storage condition: At room temperature (25 ± 2 °C/75 ± 5 % RH) and at Accelerated conditions (40 ± 2 °C/75 ± 5 % RH). The studies were carried out for different time interval up to 6 months for accelerated and 12 months at room temperature. The samples were evaluated for the parameters like visual inspection for clarity, self-emulsification time, assay, % transmittance, Z-average globule size and zeta potential. No change in physical appearance was observed during stability studies and the samples remained clear at all the storage conditions with no signs of precipitation. No significant change ($p<0.5$) in self emulsification time, assay, % transmittance, globule size and zeta Potential was observed at the end of 6 months stability study at accelerated conditions and at the room temperature for 12 months confirmed the stability of SMEDDS formulations.

11.1.7 Pharmacodynamic Study

Water maze test: Learning and intact reference memory and short term working memory

Escape latency period in learning and intact reference memory (test 1) and short-term working memory (test 2) remained unchanged for the first 4 days of testing period in all the group of Modafinil. From 5th day onwards rat learned the location of the platform in all groups of Modafinil, and there was no significant difference among them.

Water maze test on rats: Percentage time spent in each quadrant

On 6^{th} day of trial, time spent in each quadrant did not differ for Modafinil. Not a single oral group spent significantly ($p<0.05$) more time in quadrant where platform was previously placed, indicating that oral group did not learned a task on day 6th. On 7^{th} day, rats treated with Modafinil show significant difference ($P<0.05$) in time spent in quadrant where platform was previously placed compared to the saline treated rats. This demonstrated that rats treated with Modafinil learned the task by the seventh day of training, whereas rats treated with saline did not. The result did not show change on 8^{th} day despite of drug reversal. This study demonstrated noticeable improvement in learning and memory capacities with Modafinil SMEDDS by oral administration.

11.2 Conclusion

Practically insoluble drug, Modafinil leads to variable low oral bioavailability. Therefore, in the present investigation, an attempt was made to improve the solubility and thereby permeability of Modafinil and to achieve improved drug release profile by developing appropriate formulation for oral application. SMEDDS formulations were optimized by using D-Optimal Mixture design and were characterized for various parameters. Optimized batch showed good results in terms of the smaller droplet size (< 20nm), emulsification time (< 30 sec), *in vitro* drug release (>85% in 15 min), and the zeta potential values were found to be indicating good stability of formulation. SMEDDS were also stable for 6 months at accelerated condition and 12 months at room temperature. The rate and extent of drug diffusion was studied by using dialysis method and the isolated tissues of the rat, both revels that drug release from liquid SMEDDS was significantly higher than drug suspension and marketed formulation. The results demonstrate the potential of

SMEDDS as a means of improving solubility, drug release and hence the bioavailability which was confirmed by *in vivo* study. It shows that SMEDDS has achieved higher Cmax, AUC and Relative Bioavailability (Fr) compared to suspension and marketed formulation which suggests improvement in rate and extent of drug concentration in the systemic circulation. This result was also confirm by the Pharmacodynamic study which represent that the Modafinil loaded SMEDDS by oral administration gave significant improvement in learning and memory, than oral suspension and marketed ones. Thus, the study confirmed that the SMEDDS formulation can be used as a possible alternative to traditional oral formulations of Modafinil to improve its bioavailability which is helpful to manage the symptoms in Dyslexia and ADHD.

❖ Summary and Conclusion for Nasal Microemulsion

11.3 Summary

Aim of the present work was to formulate microemulsion based nose to brain drug delivery of water insoluble drug, Vinpocetine and characterized for its *in vitro*, *ex vivo* and *in vivo* potential for the management/treatment of ADHD and Dyslexia. Vinpocetine has low bioavailability around 60 % because of its first pass metabolism and after oral administration. Moreover, its absorption is also limited by its very low aqueous solubility. Hence, the novel microemulsion based approach was investigated to overcome the low penetration and aqueous solubility profile of drug along with brain targeting through olfactory region via nasal administration.

11.3.1 Analytical Method

Both UV spectroscopy and HPLC methods were developed and validated for linearity, accuracy and precision. The validation parameters for UV method were found to meet the "readily pass criteria" and % RSD were found less than 2%. The % recovery was found to be between 98-102%, showed the method was accurate to estimate Vinpocetine in the 4-20 µg/ml range. % RSD for intraday and interday of Vinpocetine at 5 different concentrations were less than 2.0% suggest that these methods have good precision and reproducibility. None of the excipients were found to be interfering in analysis of Vinpocetine, therefore UV spectroscopy method was used for study. HPLC method was developed to study and evaluate the pharmacokinetic parameters of drug. The elution was done with the flow rate of 1.5 ml/min of Acetonitrile: KH_2PO_4 (45:55) with 0.1% diethylamine at pH 3 and the retention time of Vinpocetine was found to be 10.8 ± 0.07 at 274nm of detection. The linearity range was found to be 1-20µg/ml. The % recovery (98-102%) results confirms the accuracy of the developed HPLC method. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intraday and interday of Vinpocetine at 4 different concentration levels and was found to be less than 2% RSD justifies the excellent repeatability and precision of the method. The system suitability parameters for Vinpocetine estimation by HPLC method were performed and reported. The HPLC method was further carried out at lower concentrations of Vinpocetine and was found to be linear in the range of 200-1600 ng/ml.

Moreover, the linearity was observed in the plasma in the range of 1-20 µg/ml and 200-1600 ng/ml; and also in the brain homogenate in the range of 200-1600 ng/ml.

DSC study confirms the purity of drug and FT-IR studies with drug and excipients mixture confirms the no interaction of drug with excipients.

11.3.2. Formulation and Optimization of Vinpocetine Microemulsion

Various oils, surfactants and co-surfactants were screened for the solubility study and emulsifying ability. The ratio of various components was obtained by construction of ternary phase diagram. For the optimization of the formulation, D-optimal mixture design was applied using Design Expert software version 9.0. The optimized microemulsion formulation comprised of 4% Oil (Capmul MCM C8), 56.65% Smix (Km ratio of 2:1) and 39.35% aqueous phase. Mucoadhesive and permeation enhancing (chitosan 0.5%w/v) agent was used to impart mucoadhesive strength. Globule size of the optimized microemulsion was found to be 22.19±1.28 nm and with %Transmittance of 98.03±0.61 % on 1:100 times dilution.

11.3.3 Characterization of Vinpocetine loaded ME and MME

Vinpocetine loaded microemulsion (ME) and mucoadhesive microemulsions (MME) were further characterized for thermodynamic stability testing, robustness on dilution (effect of pH and dilution factor), dye solubility, droplet size, zeta potential, pH, viscosity, % transmittance, conductance, viscosity, cloud point, assay, TEM and histopathology analysis. The ME and MME were found to be thermodynamically stable and easily dispersible. ME and MME passes the robustness on dilution test and dye solubility test. ME shows the droplet size of 19.01 ± 1.11nm with PDI value of 0.287 ± 0.034 and MME of 21.11 ± 1.56 nm with PDI 0.133 ± 0.016. Zeta potential of ME and MME systems were found to be -20.7 ± 0.44 mV and 8.46. ± 0.49 after 10 times dilution indicates the good stability of formulation. Optimized ME and MME formulation shows pH 5.51±0.07 and 5.64±0.09, within the range of nasal cavity pH. Conductance was found to be 96.21 ± 0.37 µS. Viscosity of the ME and MME were found to be 74.21 ± 0.47 µS/cm and 79.24 ± 0.35 µS/cm respectively. Assay of the microemulsion was 99.74 ± 0.13 %, shows stability of drug in formulation. Cloud point of the microemulsion was found to be at 72 ± 2 °C temperature.

ME and MME show good robustness on the dilution. Moreover, dye solubility test confirmed the devoid of any clump formation. MME shows droplet size 59.81±0.24 nm with PDI 0.272±0.04 and zeta potential -20.7 ± 0.44 mV mV which impart good stability, shows adorable pH 5.72±0.09 and conductance 177.63 ± 0.68 µS. Viscosity of MME was found to be 1190.21 ± 3.67 CP. Assay of the MME was found to be 99.59 ± 0.07 % shows stability of drug in formulation. Moreover, cloud point for MME was found to be at 68 ± 3 °C temperature. Additionally, optimized ME and MME were also checked for nasal toxicity study (Histopathology Study) and results shows both of them doesn't produce any toxicity to the nasal mucosa or no significant destruction of nasal membrane.

11.3.4 Drug Release Study

11.3.4.1 *In vitro* Drug Release Profile

In vitro drug release profile for Vinpocetine ME and MME were performed by diffusion sac method for 9 hrs in 10 % methanolic phosphate buffer saline PH 6.4. The *in vitro* release profile shows that release of Vinpocetine from MME is high as compared to drug suspension and ME. It may be due to presence of permeation enhancer in the MME formulation provides additional support for improved diffusion of drug 46.64 ± 1.31 % in 4 hr (240 min) which was higher compared to ME 34.12 ± 1.85% and for suspension it was 13.00 ± 3.64% within 4 hr.

The release kinetic pattern was studied by fitting % drug release in given time in different models of release kinetics like zero order, first order and higuchi order. Regression coefficient of all formulations in different models were compared and from the results, it was found that the release pattern of Vinpocetine from the formulations across the nasal mucosa followed by higuchi order rather than zero order and first order. This was concluded by higher regression coefficient value in curve fitting.

11.3.4.2 *Ex vivo* Drug Release Profile

Ex vivo drug release study was performed to evaluate the diffusion rate of drug from the formulation/suspension through excised sheep nasal mucosa for 4 hrs in 10 % methanolic phosphate buffer saline PH 6.4. From the results, MME formulation having additional excipients i.e. permeation enhancer (chitosan) shows improved diffusion of drug 43.39 ± 1.13 % after 4 hrs (240 min) which was significantly higher than ME 36.03 ± 0.94% and suspension it was 12.58 ± 1.94% within 4 hr.

11.3.5 *In vivo* Pharmacokinetic Study

In vivo study was performed to compare pharmacokinetic data of optimized ME and MME formulation with compared to oral suspension. Formulation of ME/MME dose was administered through nostril, while for oral suspension dose was administered through oral gavage to Male Sprague-Dawley rats. The plasma concentration vs time curve in rats after a single dose of ME/MME of Vinpocetine through nostril was compared to Vinpocetine oral suspension, the Vinpocetine concentrations in rat plasma treated with ME/MME were significantly higher than those treated with oral suspension at all the time points. The Cmax, AUC, MRT and Relative Bio-availability (Fr) for ME, MME were significantly higher than that of drug suspension. This indicated that ME and MME achieved higher amount of drug in a shorter period of time to the systemic circulation after nasal administration.

Also, intranasal administration of ME and MME showed highest Cmax (brain) when compared to orally administered suspension formulation (avoids the first pass metabolism observed by vinpocetine and because of mucoadhesive agent which open tight junction and give higher AUC).

11.3.6 Stability Studies

”The stability studies for Vinpocetine loaded ME and MME were carried out as per ICH guidelines. Chemical and physical stability of loaded formulations were assessed under different storage condition like room temperature (25 ± 2 °C/75 ± 5 % RH) and accelerated conditions (40 ± 2 °C/75 ± 5 % RH). The studies were carried out for different time interval of 0, 1, 2 and 3 months. The samples were evaluated for the parameters like Visual inspection, pH, Assay, % Transmittance, Z-average globule size and Zeta Potential. ”

“No change in physical appearance was observed during stability studies. Both the formulations remain clear at all the storage conditions with no signs of precipitation till 3 months. No significant change in pH, Assay, % T, Globule size and Zeta Potential was observed at the end of 3 months for both the formulations, indicating that they remains stable.”

11.3.7 Pharmacodynamic Study

Water maze test: Learning and intact reference memory and short term working memory

Escape latency period in learning and intact reference memory (test 1) and short-term working memory (test 2) remained unchanged for the first 4 days of testing period in all the group

of Vinpocetine. From 5th day onwards rat learned the location of the platform in all groups of vinpocetine, and there were no significant difference among them.

Water maze test on rats: Percentage time spent in each quadrant

On 6^{th} day of trial, time spent in each quadrant differ for Vinpocetine loaded ME and MME. Vinpocetine loaded ME and MME spent more time in quadrant where platform was previously placed, indicating nasal group, ME and MME treated rats started to learned on day 4th. They made a correct choice on day 6 for both test-1, learning and intact memory and test-2, short term memory in both oral and nasal formulation as well for saline. On 7th day, rats treated with Vinpocetine show significant difference ($P<0.05$) in time spent in quadrant where platform was previously placed compared to the saline treated rats in the nasal formulations. This demonstrated that rats treated with Vinpocetine learned the task by the seventh day of training whereas rats treated with saline did not. The result did not show change on 8th day despite of drug reversal. This study demonstrated noticeable improvement in learning and memory capacities with vinpocetine ME and MME by nasal administration. Improvement was best with the nasal Vinpocetine formulation than the oral formulations.

11.4 Conclusion

The poor aqueous solubility and first pass metabolism of Vinpocetine leads to variable and low oral bioavailability. Therefore in the present investigation, an attempt was made to improve the solubility of Vinpocetine and to achieve uniform drug release by developing appropriate formulation for intra nasal application which also bypass the first pass metabolism. ME and MME formulations were optimized by using D-Optimal Mixture design. Both of them were characterized for various parameters. The systems have narrow size distribution and uniform globule size. The zeta potential values were found to be indicating good stability of formulation. Histopathology study indicates that both of the formulations were nontoxic and safe to nasal mucosa. The *in vitro* and *ex vivo* drug release profiles suggested that there was uniform and improved drug diffusion in ME, MME as compared to drug suspension. TEM image also revealed that the MME formulation has uniform globules and size of droplet was in accordance with the results obtained from the Zeta sizer. The stability study for the 6 months at accelerated condition and 12 months at room temperature confirmed the stability of ME and MME formulations. It shows that ME and MME have achieved higher Cmax, AUC and Relative Bioavailability (Fr) compared to suspension

formulation which suggests improvement in rate and extent of drug concentration in the systemic circulation. The increased in AUC of the brain demonstrate selective nose to brain transport of drugs following intranasal delivery than oral delivery system. This result was also confirmed by the Pharmacodynamic study which represent that the Vinpocetine loaded ME and MME by nasal administration gave significant improvement in learning and memory than oral suspension.

Finally, the study can be concluded, that the MME is suitable for improving the diffusion as well as bioavailability of poorly water soluble drug. Furthermore, the results of the *in vivo* kinetic study illustrate that intranasal delivery a practical strategy to deliver drugs effectively to the brain. Also, the results demonstrate ME and MME a suitable delivery system to deliver drugs specifically to the brain. Therefore, it can be concluded that ME, MME can be used to increase solubility and permeation of poorly water soluble drug Vinpocetine by nasal route. Thus, the developed intranasal ME and MME would demonstrate advantage over conventional oral vinpocetine formulations in the management of brain disorders by being more brain selective with reduction in drug dose and/or frequency of dose and possibly the cost of therapy. Thus, the study confirmed that the intranasal administration of ME and MME formulation can be used as a possible alternative to traditional oral formulations of vinpocetine to improve its bioavailability which is helpful to manage the symptoms of Dyslexia and ADHD.