SUMMARY AND CONCLUSION

CHAPTER VI
SUMMARY AND CONCLUSION

6.1 ACHIEVED RESULTS / MAIN CONCLUSION

ROPINIROLE HYDROCHLORIDE LOADED IN MICROEMULSION FOR BRAIN TARGETING BY INTRANASAL DELIVERY:

The melting point of Ropinirole Hcl at found to be 249.6°C which was compiled with BP Standards. In preformulation studies, it was found that, the λ max of Ropinirole Hcl was found to be 250 nm by UV visible spectroscopy in pH 6.6 phosphate Buffer. In order to formulate Self Emulsifying Drug Delivery System it becomes a prior prerequisite to check the solubility of drug in various lipid phases, surfactant, co surfactants so that to achieve a maximum solubility to formulate a one soluble dose of the drug. The solubility of Ropinirole Hcl was carried out in various Oils, Surfactants and Co surfactants of which Castrol oil, Tween 80, PEG 400 showed maximum solubility. Selection of Oil surfactant and co surfactant is a critical step in formulation. The oil which has greater solubility is selected for final formulation. If it forms stable optically very clear emulsion with combination of surfactant and co surfactant with minimum agitation. Drug excipient compatibility studies were performed by Visual assessment, Refractive Index, pH, viscosity to indicate any problems associated with physical chemical stabilities. In order to determine the components ratios to be used in final formulation and to determine the ranges of the components (Castrol oil, Tween 80 and PEG400) pseudo ternary and ternary phase diagrams were plotted. It was determined that Smix which contains Tween 80 and PEG400 gave greater miscibility zone when used in 1:1 ratio which was selected for the final formulation and the ranges of the different components to be used in final formulation were determined by plotting a ternary phase diagrams by mixing the components in various ratios and noting down their % transmittance. From the results of solubility, emulsifying ability, concentration ranges to be used. From pseudo ternary phase diagram the Oil, surfactant and Co-surfactant were selected and total Ten formulations were developed by altering the concentrations of oil, surfactant, co-surfactant and water by maintaining the one soluble adult dose 400 mg of Ropinirole Hcl. In all the formulations the dose of the drug was maintained constant 400mg and the weight of the Oil, surfactant, Co-surfactant and water. The diffusion profiles of all
the above developed formulations were studied in pH 6.6 phosphate buffer and the formulations having best % release of drug vs time (mins) profiles were selected as final optimized studies F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 which were subjected for final evaluations. The % transmittance values of all the formulations are recorded which indicate the optimized formulations very optically very transparent which says that the emulsion formed is very fine, has a small globule size and has less interference with UV-visible light which may cause rancidity. The viscosity of all formulations F1 to F10 was found to be in range of which were within the limits of BP specifications. The refractive Index of all the formulations was again measured after one month the initial and final refractive indices didn't change more than 0.05 which indicates that there is no physical and chemical interaction between components. The all formulations F1 – F10 were checked for the temperature where the formulation develops turbidity to sort out the problems related to precipitation of drugs From the comparison of the stability data of these formulations with the initial samples, it was found that the product remained stable such at such accelerated storage conditions. No colour change or spotting was observed of these stability samples indicating that no physical degradation. For the formulations F1 to F10 diffusion studies i.e. % Cumulative drug release vs time (mins) were performed and its % cumulative drug release for all formulations was in between 83.57% to 99.93% in pH 6.6 buffer. Out of Ten formulations F2 showed the maximum % cumulative release of 99.93%. The mean particle size of all formulations were in the Range of 93.3nm to 197.2 nm which indicates that the mean particle size of the formulations are within the range. The Polydispersability Index of all formulations were in the Range of 0.254 to 0.589 which indicates that the Polydispersability Index of the formulations. The Zeta Potential of all formulations F1 to F10 were found to be in the range of 0.5mV and -19.7mV. This shows that the energy required for transfer of charge from infinite distance of dispersion medium to bilayer of emulsion globule present as dispersed phase is very high which indicates the emulsion formed is highly stable. From the report of particle size the formulation was selected and the SEM analysis was done of the same. The Scanning Electron Microscopy (SEM) analysis revealed the same that particle size ranged below 200 nm.
**In-Vivo Studies**

The result of present investigation shows that drug loaded oil in-water microemulsion for intranasal administration may be very promising approach for delivering anti-retroviral agent in order to achieve CNS targeting for the treatment of Parkinson’s disease. The physical form microemulsion in which Ropinirole Hydrochloride has given had a significant effect on the measured brain concentrations. In vivo studies data suggest that the nasal route could exploit to increase the availability of Ropinirole Hydrochloride inside the brain. However, clinical benefits of the formulation developed in this investigation will decide its appropriateness in the clinical practice for the treatment of Parkinson’s disease.

**ROPINIROLE HYDROCHLORIDE LOADED IN MICROSPHERE FOR BRAIN TARGETING BY INTRANASAL DELIVERY**

A total of 21 formulations of Ropinirole Hcl Microspheres with various polymers were formulated individually and in combination by Emulsion Solvent Evaporation technique. The formulations were subjected to evaluation parameters like particle size, surface morphology, drug entrapment efficiency, Swelling studies, In-Vitro Mucoadhesive studies and In-vitro drug release studies.

**Micromeric properties of microspheres**

All the formulations F1-F21 are evaluated for bulk density, tapped density, carr’s compressibility index and hausner’s ratio. Compressibility, i.e., Carr’s index, was found to be between 5.17 % and 22.8 % and Hausner’s ratio was found to be between 1.05 to 1.29, all the parameters indicating good flow property. The results are shown in Table.

**Percentage yield**

Percentage yield of different formulations, F1-F21, were calculated and the yield was found to be in the range of 86% to 98.9% respectively. This higher percentage yields indicates that this Emulsion Solvent Evaporation method was very useful for adoption in the formulation of Ropinirole Hcl Microspheres.

**Particle size analysis**

Particle size distribution of Ropinirole Hcl Microspheres was determined by optical microscope fitted with an ocular micrometer and stage micrometer. All the formulations of microbeads F1-F21 show uniform size distribution. The average particle size of Ropinirole Hcl Microspheres was found to be in the range of 280±3.15
µm to 535±2.28 µm. As the polymer concentration was increased, the size of the microspheres was also found to be increased.

**Drug Entrapment Efficiency (DEE)**

The entrapment process was found to be good and in the range of 61.1± 2.5% to 94.4±1.38 % for the formulations F1-F15 and 83.4±2.43 % to 95.3±2.81% for the formulations F16-F21. The percentage of entrapment was higher, 94.4±1.38% for F5 (5% Chitosan), 92.3±1.91% for F10 (3.5% Carbopol 974P) and 93.7±1.28% for F15 (3.5% Guar gum) formulation. This improved entrapment efficiency is simply due to the greater proportion of polymers with respect to amount of drug.

**Loose surface crystallography: (LSC)**

Loose surface crystallography studies were conducted for all the drug loaded formulations F1- F15 and F16-F21. Surface associated drug content of microspheres decreased with increase in the concentration of the polymer. As the polymer concentration increased from F1-F5, F6-F10 and F11-F15 it showed increased entrapment efficiencies and hence decreased surface drug contents. But in formulations with low polymer concentration the surface associated drug content was more in F1 (30.22%), F6 (32.13%) and F11 (25.01%) formulations due to the lower entrapment efficiency.

The Surface associated drug content for combination formulations, F16- F21 ranged between 10% to 18.8%.

**Swelling index: (SI)**

Swelling property was mostly affected by the concentration of polymer. As the concentration of polymers i.e, chitosan, Carbopol 974P and guar gum increases the swelling capacity increased. The swelling index for all the formulations i.e, F1-F21 was determined in pH 6.6 phosphate buffer. The Swelling index increased from F1 (175.3±8.07% )to F5(55.2±12.6%), F6(169.2±8.3%) to F10(311.4±11.25%), F11(159.8±9.13%) to F15(330.5±11.1%) in pH 6.6 buffer at the end of 2 hours. The increase in the Swelling index is due to the increase in the Polymer Concentration. The swelling capacity in pH 6.6 phosphate buffer is more which indicated greater swelling capacity in alkaline medium. The swelling index of F16-F21 formulations ranged from 270±8.34% to 350±9.6% in pH6.6 buffer at the end of 2 hours. This is due to the higher concentration of combination polymers due to the combinations of polymers. The results are showing in table 30.
In vitro drug release studies:
The invitro drug release studies were conducted for all the formulations i.e, F1-F21 in 250ml phosphate buffer pH 6.6 for 12 hours. The percentage of drug release for formulations F1-F5 were found to be in the range of 86.4 ± 1.22 % to 98.9± 0.54% The maximum drug release was found to be 98.9±0.54 % in formulation F1 in 12 hours due to the initial burst release but the formulation F5 showed 86.4± 1.22% drug release in 12 hours showing sustained release due to increase in polymer concentration i.e, 5% Chitosan. Similarly for the formulations F6- F10 the drug release was found to be in the range of 85.8±0.43 to 96.8±0.55% and F10 showing 85.8±0.43% drug release as sustained manner in 12 hours when compared to the other formulations. This is due to increase in the polymer concentration i.e, upto 3.5% Carbopol 974P. Similarly for the formulations F11-F15, the percentage drug release was found to be in the range of 82.7±0.23% to 95.5±0.83 % and F15 showing 82.7±0.23% drug release as sustained manner in 12 hours as compared to the other formulations. So this is due to increase in the polymer concentration upto 3.5% Guar gum. The comparative dissolution studies were conducted for the formulations F5, F10 and F15. Among them, F15 showed 82.7± 0.23% drug release in 12 hours in a sustained manner. So the formulation F15 is suitable for sustained release of metoprolol succinate and also showed the better percentage yield, encapsulation efficiency and swelling properties.

The invitro drug release for the formulations F16 & F17 are 87.5% and 82.9% respectively in 12 hrs. This showed sustained release due to the combination of Chitosan and Guar gum in Polymer Concentrations LL (3.5%) and HH (5%) respectively. The drug release for the formulations F18 & F19 in 16 hrs are 89.1% and 83.6% respectively. This showed drug release in a sustained manner due to the combination of polymers Chitosan and Carbopol 974P in Concentrations LL (3.5%) and HH (5%) respectively. The drug release for the formulations F20 & F21 in 16 hrs are 85.6% and 81.2% respectively. The sustained release in these formulations are due to the combination of polymers Carbopol 974P and guar gum in concentrations LL (3.5%)and HH (5%). The comparative dissolution was run for the formulations F17,F19, F21. Among them F21 showed 81.2% drug release in 12 hrs in a sustained manner. So the formulation F21 is also suitable for sustained release of Ropinirole.
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Hcl using combination of polymers, Carbopol 974p and Guar gum. The results of *invitro* dissolution studies are shown in the Table & Figure.

**Drug release Kinetics:**

The *invitro* dissolution data for formulations F1-F21 were analysed for different kinetic models in order to find out which drug release mechanism it follows. The values of correlation (r) were calculated and were found to be more linear for first order release as compared to zero order. The kinetic data was best fitted to Higuchi model and good regression coefficients were observed. The results are shown in Table. The kinetic model graphs of F15 formulation and F21 formulation are shown in Figure.

**Scanning Electron Microscopy: (SEM)**

Surface morphology and internal cross sectional structure of the microspheres were investigated with scanning electron microscope. The microspheres were smooth, spherical and discrete particles. Very less particulate matter of the drug were seen on the surface of the microspheres indicating uniform distribution of the drug in the polymer network.

**Stability studies**

At the end of stability studies, the microspheres were checked for any changes in physical stability, size, shape, drug content and release profile. Selected formulations like F15 & F21 microsphere were subjected to exhaustive stability testing at 25±2°C 60±5% RH for 1st & 2nd month and 40±2°C 75±5% RH for 3rd months. Samples were withdrawn at 1, 2 and 3 months period according to ICH guidelines. The both formulations (F15 &F21) did not show any changes in physical stability, size, shape, drug content and release profile at intermediate conditions.

**In-Vivo Studies**

The present study has been satisfactorily attempted to formulate a mucoadhesive microspheres system of an antiparkinsons drug like Ropinirole Hydrochloride for intranasal administration with a view of enhancing bioavailability of the drug. Assessment of AUC showed that the relative bioavailability was found to have significantly increased 4.51% and 4.90% for F15 and F21 respectively. From all the parameters studied, it can be concluded that combination of Carbopol 974P and guar gum is better mucoadhesive polymer for the formulation of mucoadhesive
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microspheres of Ropinirole Hydrochloride for intranasal administration. Thus, the formulated microspheres seem to be a potential candidate as intranasal controlled drug delivery system for treatment of Parkinson’s disease.

6.2 FUTURE DEVELOPMENT AND SCOPE

The present investigation on Ropinirole Hydrochloride drug loaded oil in-water microemulsion and microsphere for intranasal administration may be very promising approach for delivering antiretroviral agent in order to achieve CNS targeting for the treatment of Parkinson’s disease.

The physical form of delivery system in which Ropinirole Hydrochloride has been given had a significant effect on the measured brain concentrations.

However, clinical benefits of the formulation developed in this investigation will decide its appropriateness in the clinical practice for the treatment of Parkinson’s disease.

This work is now open for other researchers and community pharmacist to explore further dimensions on the said path.