SUMMARY AND CONCLUSIONS

The oral route is by far the most convenient one for drug administration. However, for oral administration, the low concentration gradient between the gut and blood vessel due to the poor solubility of the drug leads to a limited transport, consequently influencing the oral absorption. Recently, drug delivery research mainly focuses on nanotechnology based strategies for drugs with low solubility to improve their bioavailability. Nanoparticulate technology such as pure drug nanoparticles and nanoemulsion has proven its competence for numerous drugs with low solubility in order to improve their therapeutic performance. The ability to formulate poorly-water soluble compounds as nanometer sized particles can have a dramatic effect on performance, such as enhancing bioavailability, eliminating food effects, allowing for dose escalation and hence improving efficacy and safety. Thus, the present investigations focused on nanosizing approaches such as nanosuspension and nanoemulsion.

The present investigation was aimed at the development of nanoparticulate delivery systems for oral administration of Simvastatin and Entacapone to improve their bioavailability. Nanoparticulate delivery systems such as drug nanoparticles and nanoemulsions were prepared and optimized by using factorial design. The drug nanoparticles were prepared by media milling and supercritical method while nanoemulsions were prepared by ultrasonication method.

It was envisaged that drug nanoparticles and nanoemulsions will improve solubility, dissolution rate and permeability which will ultimately increase absorption and hence bioavailability of these poorly water soluble drugs.

9.1 Simvastatin

Nanosuspensions

Simvastatin nanosuspension (SNS) was prepared by media milling method using zirconium oxide beads. The process and formulation parameters were optimized systematically. After preliminary experiments, critical parameters were identified. The important parameters such as media milling volume, surfactant concentration were optimized by factorial design.
The optimized formulation contained 0.5% w/v drug, 1% Tween 80 and 100% milling media volume and milling was carried out for 14 hr. The optimized nanosuspensions were evaluated for particle size, zeta potential, saturation solubility, surface morphology, drug content, in vitro drug release, DSC, XRD and stability studies.

Simvastatin nanosuspension (SNS) showed particle size of 250 ± 09 nm (PI 0.238) and zeta potential was -27.1 ± 3.5 mV. This indicates nanonization of Simvastatin due to media milling and resulted in improved dissolution characteristics. The increase in solubility in case of SNS was almost 3.5 folds higher than the bulk Simvastatin.

The TEM micrographs of SNS confirmed that the milling process was effective in converting bulk Simvastatin particles into the submicron range. Nanosized particles with irregular shape and without any tendency of aggregation were observed indicating change in morphology as that of plain drug which showed needle shaped crystals. XRD and DSC studies showed change in crystallinity of Simvastatin compared to plain drug. In XRD studies, the absence of major peaks of Simvastatin for SNS confirmed formation of amorphous product which might lead to enhanced solubility of the drug in case of SNS. DSC scan of bulk Simvastatin sample showed a single sharp endothermic peak at 140 °C ascribed to the melting of the drug but in SNS, disappearance of melting endothermic peak was observed which indicated substantial crystalline change of Simvastatin due to nanosizing.

Drug content of SNS formulation was found to be 98.53±1.65 % which indicated suitability of method for particle size reduction. The in vitro release of nanosuspension was compared with plain drug. The drug release of 12.35±0.44 % was observed in case of plain drug at 2 hr while the drug release in case of nanosuspension formulation was 39.96±0.34 %. Similarly, at the end of 8 hr, 16.69±0.98 % of drug was released in case of plain drug while the drug release of SNS was 52.4±0.84 %. This increase in release rate can be attributed to increase in the surface area after nanosizing the crystals. The drug release from SNS followed Peppas–Korsmeyer ($r^2=0.9711$) and followed fickian diffusion mechanism. The SNS was found to be stable for the period of 3 months at room temperature and cold conditions. Thus, nanosizing approach could play important role in improving solubility of Simvastatin which will ultimately lead to enhancement in its bioavailability.
**SCF formulation**

Another approach used for size reduction of Simvastatin was supercritical methods. The plain drug particles of Simvastatin were prepared by supercritical antisolvent method using DCM as organic solvent. The process and formulation parameters were optimized to obtain minimum particle size on trial and error basis. The product obtained by supercritical method (SCF formulation) was evaluated for particle size, saturation solubility, surface morphology, flow properties, drug content, in vitro drug release, DSC, XRD and stability studies.

Simvastatin SCF formulation showed particle size of 16.54±0.38 µm (PI 0.546). This indicated reduction in particle size of Simvastatin but nanosizing did not occur. The saturation solubility in case of SCF formulation (0.614 ± 0.078) was slightly higher than plain drug (0.548± 0.062 mg/ml).

The SEM micrographs of SCF formulation confirmed that the particle size was reduced in case of SAS process but there was no conversion of bulk Simvastatin into the nanosized product. The presence of needle shaped crystals was observed. DSC XRD studies were also performed to confirm crystallinity of SCF formulation. In XRD studies of SCF formulation, all the peaks of Simvastatin were retained indicating no change in crystallinity of Simvastatin after SCF processing. This was also confirmed from DSC studies which showed that endothermic peak of Simvastatin was retained in case of SCF formulation.

Drug content of SCF formulation was found to be 97.94±1.65 % indicating suitability of the methods for particle size reduction. The release profile of SCF formulation was compared with plain drug. The drug release of 12.35±0.44 % and 15.8±0.94% was observed in case of plain drug and SCF formulation at 2 hr respectively. At the end of 8 hr, 16.69±0.98 and 18.71±0.95% of drug was released in case of plain drug and SCF formulation respectively. The drug release from SCF followed Korsmeyer-Peppas ($r^2=0.9854$) and followed fickian diffusion mechanism. This indicates that there was slight increase in drug release from SCF formulation. The product obtained in SCF method was very fluffy with different physicochemical properties and it also showed reduction in particle size compared to plain drug, hence was evaluated for in vivo performance.
Nanoemulsions

The nanoemulsion formulation of Simvastatin was prepared by ultrasonication method. Capryol 90 was selected as oil phase based on solubility of the drug. The process and formulation parameters were optimized systematically, in which initially process parameters such as high speed mixing time, sonication time were optimized and then selection of surfactants was done. After preliminary experiments, important parameters such as oil percentage and ratio of surfactants were optimized by factorial design. The optimized formulation contained 15% v/v Capryol 90, 2% w/v phospholipon 90, 1% w/v Pluronic F68, 1.8% w/v drug and 85% v/v distilled water. The nanoemulsions were evaluated for particle size, zeta potential, pH, surface morphology, drug content, viscosity, in vitro drug release and stability studies.

The average particle size diameter of the Simvastatin loaded nanoemulsions was found to be 132±9 nm. The polydispersity index (PI) of was low (below 0.2) and this unimodal distribution indicates uniformity in globule size. The TEM image showed that the globules were spherical, possessed diameter ranging from 120-180 nm and had smooth surface. The drug content was found to be 98±1.2 %. The pH of the nanoemulsions was found to be 4.8±0.2. The viscosity of the nanoemulsion was found to be 2.020±0.01 cp. The nanoemulsion formulation was not viscous.

The in vitro release studies showed significant increase in drug release as compared to plain drug suspension. Plain drug suspension (PD) showed only 12.35±0.44% drug release in 2 hr while nanoemulsion (NE) showed 22.37±0.92% drug release which was approximately double. At the end of 8 hr, plain drug suspension showed only 16.69±0.95% drug release while nanoemulsion formulation showed 33.81±0.98% drug release. The drug release from SNE followed Korsmeyer-Peppas ($r^2=0.9387$) and followed fickian diffusion mechanism. This could be attributed to enhanced solubility and dissolution rate of Simvastatin which in turn can be due to low globule size and surface properties of the nanoemulsion. The nanoemulsion formulation showed physical stability for a period of 3 months at room temperature and cold conditions in terms of drug content and globule size.
Pharmacodynamic studies of Simvastatin formulations

The clinical effects of Simvastatin would be evident by lowering of total cholesterol (TC) level and increment in levels of high-density lipoprotein (HDL). This pharmacodynamic effect is reported to be dose dependent and hence, was used as a basis for the comparison of in vivo performance of the prepared formulations and the plain drug suspension.

Induction of hyperlipidemia was confirmed from the increase TC level and TG levels of control sample. There was significant increase in TC and TG levels after administration of the high fat diet at 4, 7 and 14 days of the treatment in control group. There was reduction in TC level for SNS (7.88±2.43), SNE (9.59±3.55) and SCF (6.74±3.26) formulation as compared to PD at 4 days. At 7 days of administration, SNS showed significant reduction in TC level (23.05±6.73 %) as compared to plain drug suspension (2.39±1.66 %) (P<0.05). SNE also showed significant reduction in TC level (15.34±5.63%) as compared to PD at 7 days of administration (P<0.05) while for SCF formulations reduction in TC level was 8.11±4.20 % at 7 days of administration. At 7 days the percent protection in term of TC reduction offered by SNS (207.8%), SNE (171.8%) was much higher than that of PD (111.3%) and SCF formulation (137.9%). At 14 days also, higher reduction in TC level was observed for SNS, SNE as compared to PD. These results indicate that the prepared formulations (SNS, SNE and SCF) were more efficient in controlling TC level as compared to PD and this can be attributed to enhanced dissolution rate of the formulations leading to improved bioavailability. Among all these formulations, SNS showed higher reduction followed by SNE and SCF formulation.

Similarly, the reduction in TG level in case of all formulations was higher at all intervals as compared to plain drug suspension. SNS showed significant reduction in TG level (7.54%) as compared to plain drug suspension at 4 days of administration (p<0.05). SNE showed significant reduction in TG level (16.70%) as compared to plain drug suspension at 14 days of administration (p<0.05). The reduction in TG levels was higher at 14 days for SNE while it was higher at 4 days for SNS and SCF formulation. Also, there was higher increase in HDL levels for SNS, SNE and SCF than PD. This reduction in TG level and increase in HDL level of these formulations compared to PD could be due to enhanced bioavailability of Simvastatin from the formulations.
Pharmacokinetic studies of Simvastatin formulations

After oral administration, nanosized formulations i.e. SNS, SNE and SCF formulation exhibited higher plasma level concentration compared to PD. The AUC_{last} for SNS was found to be $44.11 \pm 6.45$ ng.h/ml, which was significantly higher than PD which showed AUC_{last} of $16.73 \pm 6.56$ ng.h/ml ($P<0.001$, one way ANOVA followed by Bonferroni's multiple comparison test). The AUC_{last} of SNE (61.75±8.03) was also significantly higher than that of PD ($P<0.0001$), while it was non-significant for SCF (30.29±6.7) formulations. Among all these formulations, SNE showed highest AUC_{last} followed by SNS and SCF formulation. When the C_{max} was of these formulations were compared, significant improvement in C_{max} in case of SNS compared to PD was observed ($P<0.0001$, one way ANOVA followed by Bonferroni's multiple comparison test). The C_{max} value was significantly higher in case of SNE ($P<0.001$), while there was no significant difference in case of SCF formulation.

The relative bioavailability of the SNS and SNE were 263.6 and 369.0 % respectively with respect to plain drug suspension. Thus, there was about 2.6 and 3.7 times increase in bioavailability of Simvastatin for SNS and SNE respectively and it could be attributed to enhanced surface area due to nanosizing and improved dissolution rate. The relative bioavailability of SCF formulations was also 1.8 times as compared to plain drug which could be attributed to decreased particle size of SCF and changed physical properties of the formulation. Among all the formulations studied, SNE was the best with nearly 3.7 times increase in bioavailability followed by SNS and SCF.

The results obtained in this pharmacokinetic study are well supported by the pharmacodynamic studies, which showed enhanced hypolipidemic activity of nanosized formulations compared to plain drug as indicated from % protection offered in hyperlipidemia. Thus, it can be inferred that improvement in bioavailability of Simvastatin is possible with nanosizing approaches such as nanosuspension and nanoemulsion.
9.2 Entacapone Nanosuspension

Entacapone nanosuspension (ENS) was prepared by media milling method using zirconium oxide beads. In preliminary experiments, milling time, type of milling media, selections of surfactant and drug concentrations were optimized. The important parameters such as media milling volume, surfactant concentration were optimized by factorial design. The optimized formulation contained 2.0% w/v drug, 1.0% Pluronic F127 and 100% milling media volume and milling was carried out for 12 hr. The nanosuspensions were evaluated for particle size, zeta potential, saturation solubility, surface morphology, drug content, in vitro drug release, DSC, XRD and stability studies.

Entacapone nanosuspension (ENS) was successfully prepared by milling with particle size of 231 ± 1.2 nm (PI 0.211) after 12 hrs. The PDI value of Entacapone nanosuspension was below 0.25 indicating a narrow size distribution of the milled suspension. The zeta potential of the prepared ENS was -22.8 ± 3.5 mV. The saturation solubility of ENS was 2.208±0.125 mg/ml as compared to Entacapone plain drug 0.315±0.036 mg/ml, indicating significant enhancement in solubility. The increase in solubility in case of ENS was almost 7.0 folds higher than the plain Entacapone.

The appearance of ENS was compared with plain drug suspension by TEM and SEM studies. Entacapone plain drug exhibited large aggregates of needle shaped crystals while ENS showed nanosized eclipse shaped particles with smooth surface and particles were non-aggregated. TEM micrographs further confirmed that the milling process was effective in converting the Entacapone plain drug particles into the submicron range. The XRD results showed significant reduction in crystallinity of Entacapone in case of ENS as compared to plain drug. DSC scan of Entacapone plain drug showed sharp endothermic peaks at 155 and 159 °C related to the melting of the drug. This peak was not observed in case of ENS, which indicated significant reduction in crystallinity of the drug. XRD and DSC studies proved change in crystallinity of Entacapone by nanosizing.

Drug content of ENS was found to be 99.03±0.305 %. In vitro release of ENS was studied in pH 1.2 and pH 7.2 phosphate buffer. In 0.1 N HCl, within initial 10 min, 16.33 % of drug was released in case of ENS and the corresponding release for plain drug was 9.17%. Similarly
at 8 hr, 88.23 % of drug was released in case of ENS and the corresponding release for plain drug was 62.12%. At pH 7.2 also, within initial 10 min 12.27 % of drug was released in case of ENS and the corresponding release for plain drug was 10.47%. This increase in drug release could be attributed to the increased saturation solubility and surface area due to nanosizing. The higher release in case of PD could be due high solubility of drug with increased pH. The release profile was fitted in different kinetic model to find out release rate and mechanism. The ENS formulation showed value of ‘n’ between 0.5-10 indicating anomalous transport mechanisms at both pH conditions. The formulation was found to be stable in term of particle size and drug content for 3 months at room temperature and cold conditions.

**Solid dispersion particles by SAS method**

Nanosizing of Entacapone plain drug was done by SAS method, but nanonization of the plain drug was not significant, hence solid dispersion particles of Entacapone were prepared by SAS method for improving its dissolution.

Entacapone solid dispersion particles (E-SDP) were prepared by using hydrophilic polymer, HPMC K 15 and Pluronic F68. The precipitation conditions in SAS methods were optimized based on product yield and particle size obtained.

The E-SDPs were evaluated for particle size, zeta potential, surface morphology, drug content, in vitro drug release, DSC, XRD and stability studies.

The particle size of optimized E-SDPs was found to be 560±14 nm (PI 0.492). The results showed the E-SDPs obtained by supercritical antisolvent method had particle size in nanometer range indicating suitability of method for nanosizing. The zeta potential of E-SDPs was found to be -12.3 mV. The incorporation efficiency of E-SDPs was found to be 20.88±2.35 % indicating the SC CO\text{$_2$}-solvent system does not always dissolve the two ingredients to the same extent.

The appearance of E-SDPs was compared with plain drug suspension by TEM studies. Entacapone plain drug exhibited large aggregates of needle shaped crystals. It can be confirmed from TEM studies that morphology of bulk drug was changed by SAS method and the reduction in particle size to nanometer size was observed. The surrounding matrix
around the particles indicates the eroded HPMC matrix. The change in crystallinity of Entacapone in E-SDPs was studied by XRD and DSC studies. In case of E-SPD, all the major peaks associated with plain drug had disappeared indicating complete reduction in crystallinity. In this case, DSC scan of bulk Entacapone sample showed sharp endothermic peaks at 155 and 159 °C related to the melting of the drug. Also these characteristics peaks of Entacapone were not observed for E-SDPs indicating formation of amorphous product. Thus, a combined XRD and DSC study proved that crystallinity of Entacapone was changed by formulating as E-SDPs.

In vitro release of E-SDPs was studied at pH 1.2 and pH 7.2 phosphate buffer. The drug release of Entacapone was significantly improved in case of E-SDPs in 0.1 N HCl. Within initial 10 min, 23.27% of drug was released in case of E-SDPs and the corresponding release for plain drug was 9.17% which could be attributed to the increased solubility due to nanosizing and solubility enhancing effect of HPMC. At pH 7.2, 20.91% of drug was released in case of E-SDPs at 10 min while the corresponding release for plain drug was 10.47%. This could be due nanosizing and enhanced hydrophilicity of the formulation. E-SDPs showed value of ‘n’ of 0.47 indicating diffusion controlled mechanism at pH1.2 while at pH 7.2 value of ‘n’ of between 0.5-10 indicating anomalous transport mechanisms. The formulation was found to be stable in term of particle size and drug content for 3 months at room temperature and cold conditions.

Nanoemulsion

The Entacapone nanoemulsion (ENE) was prepared by ultrasonication method. Capmul MCM was selected as oil phase based on solubility of the drug. The process and formulation parameters were optimized systematically, in which initially process parameters such as high speed mixing time, selection of surfactants, sonication time were optimized. After preliminary experiments, important parameters such as oil percentage and ratio of surfactants were optimized by factorial design. The optimized formulation contained 15% v/v Capmul MCM, 0.5% w/v phospholipon 90, 0.5% w/v Pluronic F68, 0.12% w/v drug and 85% v/v distilled water. The ENE were
evaluated for particle size, zeta potential, pH, surface morphology, drug content, viscosity, in vitro drug release and stability studies. The average particle size diameter of the ENE was found to be 120.8±1.9 nm. The polydispersity index (PI) of ENE was 0.144 (below 0.2) and this unimodal distribution indicates uniformity in globule size. The zeta potential of ENE was found to be -20.6±0.8 mV. The drug content was found to be 99.4±0.8 %. The pH of the nanoemulsions was found to be 5.5±0.2 indicating suitability for oral administration. The viscosity of the nanoemulsion was found to be 1.63 ±0.02 cp.

Morphology and droplet size of the ENE was evaluated by TEM. The TEM image showed that the globules were almost spherical, and had diameter ranging from 120-150 nm. The globules were segregated and showed no tendency of aggregation.

The in vitro release profile of ENE and plain drug suspension (PD) was studied at pH 1.2. The in vitro release studies showed increase in drug release for ENE as compared to PD at pH 1.2. Plain drug suspension showed only 25.05±0.44% drug release in 60 min while nanoemulsion formulation showed 31.77±0.92% drug release. At 8 hr, Plain drug suspension showed 62.12% drug release while nanoemulsion formulation showed 80.33±0.92% drug release indicating improved drug release which could be attributed to enhanced solubility of Entacapone and dissolution rate which in turn can be due to low droplet size and surface properties of the nanoemulsion. ENE formulations showed value of ‘n’ between 0.5-1.0 indicating anomalous transport mechanisms at both pH conditions. ENE showed value of ‘n’ between 0.5-1.0 indicating anomalous transport mechanisms at pH 1.2. The nanoemulsion formulation found to be stable in term of globule size and drug content for the period of 3 months at room temperature and cold conditions.

Pharmacokinetic studies:

After oral administration, all nanosized formulations (ENS, ENE and SDP) showed higher plasma concentration as compared to PD. The AUC\textsubscript{last} of ENS (641.70±37.59,), ENE (1032.61±39.88) and E-SDP (1702.27±47.77) were found to be significantly higher as compared to AUC\textsubscript{last} of PD (419.58±30.92). There was significant enhancement for ENE and E-SDP as compared to PD (P<0.001, one way ANOVA followed by Bonferroni’s multiple
comparison test). Similarly, all these nanosized formulations showed significantly higher AUC_{total} values as compared to PD. The C_{max} of ENE and E-SDP were 643.87±47.2 and 1894.48±71.7 ng/ml respectively, which was significantly higher as compared to AUC_{last} of PD (407.64±28.87) (P<0.001, one way ANOVA followed by Bonferroni’s multiple comparison test). However, the difference in ENS and PD was not significant. Overall, these results indicate improvement in bioavailability which was attributed to increased dissolution rate and bioadhesion. The presence of a surfactant in the nano-formulation causes changes in membrane permeability by the inhibition of an apically polarised efflux system, which could lead to enhancement of the oral absorption. Thus, formulation composition might have attributed to permeation enhancement in case of ENE and E-SDP.

The relative bioavailability of the nanosized formulations with respect to PD was calculated to compare the in vivo performance of these formulations. The relative bioavailability of the ENS, ENE and E-SDP were 152.9, 246.1 and 405.7% respectively with respect to PD. Thus, there was 1.5, 2.4 and 4.0 times increase in bioavailability of Entacapone for ENS, ENE and E-SDP respectively and it could be attributed to enhanced surface area due to nanosizing and improved dissolution rate. The enhancement in dissolution rate of Entacapone (at pH 1.2) was also supported by in vitro release studies. In case of E-SDP, complete amorphization was observed which might have contributed to enhancement in bioavailability as compared to PD and ENS. Other parameters i.e. permeability might have contributed in case of ENE and E-SPD. Thus, overall it can be concluded that nanosizing of Entacapone by these approaches results in improvement in its oral bioavailability.

9.3 Conclusions:
In the present investigations, nanoparticulate delivery systems such as nanosuspensions, nanoemulsions and SCF formulations by SAS method were prepared for oral administration of Simvastatin and Entacapone to improve their bioavailability. In the in vivo studies, the relative bioavailability of the SNS, SNE and SCF were 263.6%, 369.0% and 181% respectively with respect to plain drug suspension for Simvastatin. Also in pharmacodynamic studies, better reduction in TC levels was observed as compared to plain drug in case of SNS, SNE and SCF formulation. Similarly, the relative bioavailability of the
ENS, ENE and E-SDP were 152.9, 246.1 and 405.7% respectively with respect to PD for Entacapone. The improvement in bioavailability of Simvastatin and Entacapone was supported by the physicochemical characteristics of the nanosized formulations. Enhanced dissolution was observed for these systems compared to conventional drug suspension.

The results of the present investigations conclusively indicate the enhancement in bioavailability of the drugs, Simvastatin and Entacapone, when administered as nanosized formulations through oral route. Hence, the developed nanosized formulations of Simvastatin and Entacapone can be potentially useful in clinical treatment of hypertension and Parkinson’s disease respectively. Thus, these formulations hold promise as better alternative to the conventional dosage forms. However, further investigations in human beings under clinical conditions are necessary before they can be commercially exploited.