SUMMARY

The oral delivery route is commonly recognized as the most preferred and convenient for the administration of drug formulations. The hydrophobic drugs belonging to Class II of the biopharmaceutical classification system, were the dissolution rate control and therefore, determines the rate and degree of absorption. Approximately, 40% of new chemical entities entering development programs had very low aqueous solubility or oral bioavailability to bring about satisfactory therapeutic efficacy. Thus, one of the major current challenges facing the pharmaceutical industry involves the development of strategies to improve the aqueous solubility, dissolution rate and bioavailability of poorly soluble drugs.

Simvastatin is a poorly soluble lipid lowering agent, which is used for the treatment of primary hypercholesterolemia. Water solubility of simvastatin is very low, and poorly absorbed from the gastrointestinal tract. Another Class II drug of lovastatin is a cholesterol reducing drug, has great selectivity for the liver and the extensive first-pass effect results in low systemic bioavailability, leaving only a small amount (<5%) in the systemic circulation.

Therefore, it is very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its bioavailability. Improvement of the aqueous solubility in such a case is a valuable goal that leads to enhancing therapeutic efficacy. Considerable efforts have been directed at increasing the solubility of these hydrophobic compounds by creating nanoparticles formulations with high surface to volume ratios.

The present study was carried out to develop nanoparticles of simvastatin and lovastatin in order to enhance solubility, dissolution and bioavailability by decreasing the particle size of the drug.

In part I, Preformulation studies revealed that simvastatin and PLGA were compatible without any significant changes in chemical structure of simvastatin. From FTIR spectral analysis, it was concluded that there was no interaction between the drug, polymer and physical mixture as the principle peaks of the drug were found unaltered in the FTIR spectra of drug, polymer and physical mixture.

The particle size of all drug loaded nanoparticles formulation batches were found in the range 100-300 nm. The smallest (122 ± 1.52 nm) particle size was obtained for batch PS6, while largest (293 ± 2.64 nm) for batch PS7. Polydispersity index of prepared NPs batches were found in the range of 0.4508 to 0.9669.

Entrapment efficiency of batches under investigation were in the range of 70.0-97.0 %, batch PS6 showed entrapment efficiency 85.43 ± 0.49 %, whereas PS3 batch showed lowest entrapment efficiency 70.59 ± 0.62 %.
Zeta potential of all nanoparticles batches were found in the range (-12.04 ± 0.022) (Mv) to -23.32±0.01 (Mv). Zeta potential of PS6 optimized batch was (-23.32±0.01) (Mv), i.e. near to range, which indicates good physical stability of nanoparticles.

Cumulative drug release for all formulations batches PS1–PS9 were found to be 77.24± 0.317% to 96.53 ± 0.501% respectively, after 60 min. Cumulative drug release for PS6 was found to be 96.53 ± 0.501% respectively, after 60 min. The drug release follows Matrix and first order release kinetics mechanism. The nanoparticles obtained after freeze drying process were in the form of powder. Before freeze drying the particle size was 122 ± 1.52 nm, whereas after freeze drying it became 135 ± 1.52 nm.

The saturation solubility of simvastatin pure was 16.82 ± 0.73 μg /ml and SV-loaded nanoparticles resulted in increase in solubility after 48 h (81.58 ± 1.60 μg/ml) in comparison with SV pure drug, i.e increased solubility approximately 5 fold.

The % process yield of all nanoparticles batches were found to be from 81.56 ± 0.59 % to 89.62 ± 0.93 %. The result reveals that % loss of all batches was very negligible during processing of freeze drying.

The % drug content and percentage recovery of optimized PS6 batch was found to be 94.48 ± 0.44 %. The result reveals that, low loss of drug content of optimized PS6 batch during freeze drying.

The DSC thermograms studies show that the crystallinity of the drug has been reduced significantly in the nanoparticles. Hence, it could be concluded that the prepared PLGA nanoparticles loaded simvastatin was present in the amorphous phase and may have been homogeneously dispersed in the PLGA matrix.

The X- ray diffraction spectrum of pure simvastatin SV showed that the drug was of crystalline nature as indicated by numerous, distinct peaks. The nanoparticles prepared with PLGA was characterized by less intensity of the diffraction peak, when compared to that of simvastatin.

The SEM studies revealed that simvastatin pure drug consisted of a mixture of large crystals, indicating its crystalline nature. However, the prepared simvastatin loaded PLGA nanoparticles of batches were nearly spherical shape with a relatively uniform size in diameter and no drug crystals were present.

The Transmission electron microscopy (TEM) studies revealed that freeze dried optimized batch PS6, was spherical shape and uniform size.

In vitro drug release after 60 min. dissolution studies, the results revealed that nanoparticles tablet formulation was found to be increased i.e. 91.96±0.064% as
Summary and Conclusion

Chapter 8

Formulation and Evaluation of Nanoparticles For Better Drug Bioavailability

compared to pure drug tablet 26.95±0.022%, marketed tablet 43.91±0.018%. The best fit peppas model mechanism of PS6 nanoparticles tablet formulation.

Stability studies results indicate that there was no evident change in the physical appearance but particle size increased and drug content decreased of PS6 optimized batch formulations after subjecting them to stability studies.

In vivo antihyperlipidemic activity performed on albino rats, after 21 days of treatment, results shows that plasma CH and TG levels were significantly lower (84.10± 0.026 mg/dl, 168.60± 0.130 mg/dl respectively) (p < 0.001) and HDL-CH levels were significantly higher (45.58± 0.081 mg/dl, p = 0.007) in TTG compared to RTG. Thus, TTG showed a significantly better in vivo performance than RTG in terms of plasma lipid profile parameters.

The pharmacokinetic parameters of simvastatin nanoparticles, simvastatin pure and simvastatin marketed tablets were compared in Albino rabbits. Cmax of simvastatin nanoparticles was found to be 63.35 ± 0.195 ng/ml, whereas Cmax value for the drug suspension and marketed tablet formulation was found to be 34.00 ± 0.100 ng/ml and 46.91± 0.194 ng/ml respectively. (p < 0.001). T max of simvastatin nanoparticles was 2 hrs, whereas it’s value for the drug suspension and marketed tablet formulation was 1 h respectively. (p < 0.001). AUC (0 – 8 h) value for the simvastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 235.36 ± 0.101(ng/ml × h), 152.72 ± 0.20 (ng/ml × h) and 189.22± 0.23 (ng/ml × h) respectively. (p < 0.001) The results revealed that relative bioavailability (Fr %) was increased as compared to oral control group standard I & II. It was found that relative bioavailability was 119.55 % and 151.80 % respectively. (p < 0.001) The in vivo study results reveal that simvastatin nanoparticles show better bioavailability than drug suspension and marketed formulation. Stability studies results indicate that after the 1, 2 and 3 months accelerated stability studies, no morphological changes were observed but particle size increased, drug content and % cumulative release decreased in nanoparticles dispersion, freeze dried nanoparticles. After the 1, 2 and 3 months accelerated stability studies reveals that % cumulative release decreased in pure tablet, marketed tablet and freeze dried nanoparticles tablets.

In part II, Preformulation studies revealed that lovastatin and chitosan were compatible, from FTIR studies it can be seen that the fundamental peaks of lovastatin are retained. Results show that there was no chemical interaction between lovastatin and chitosan used in the formulation hence, can be used in the formulation of nanoparticles.

The particle size of all drug loaded nanoparticles formulation batches were found in range 200- 700 nm. The smallest (292 ± 3.51 nm) particle size was obtained for batch CL4, while largest (702 ± 2.51 nm) for batch CL9. Polydispersity index (PI) of prepared NPs batches were found in the range 0.5376 to 0.9188 and optimized batch was 0.5376, which indicates particle size spherical and uniform.
Entrapment efficiency of batches under investigation was in the range of 94.03 ± 0.025%-98.85 ± 0.035 %, Optimized batch CL4 showed highest entrapment efficiency 98.85 ± 0.035 %, whereas CL9 batch showed lowest entrapment efficiency 94.03 ± 0.025%.

Zeta potential of all nanoparticles batches were found to be in the range +11.27± 0.020 (Mv) to +48.50 ±0.072 (Mv). Zeta potential of CL4 batch was (+27.10 ± 0.065) (Mv), means near to range, which indicates good physical stability of nanoparticles.

In vitro drug release for all formulations batches CL1- CL9 were found to be 77.98 ± 0.026 % to 91.06 ±0.026% respectively, after 60 min. Cumulative drug release for CL4 was found to be 91.06 ±0.026% respectively, after 60 min. The drug release follows Hixon crowel & first order release kinetics mechanism.

The nanoparticles obtained after freeze drying process were in the form of powder. Before freeze drying the particle size was (292 ± 3.51 nm), whereas after freeze drying it becomes (304 ± 2.64nm).

The saturation solubility studies showed increased solubility for an amorphous state of lovastatin loaded CS nanoparticles compared to lovastatin. The solubility of lovastatin was (62.17 ± 0.020 μg /ml).Lovastatin -loaded nanoparticles, increase in solubility after 48 h (237.4 ± 0.041 μg/ml) in comparison with lovastatin pure drug, i.e increase solubility approximately 5 fold.

The % process yield of CL4 batch was higher (90.15 ± 0.734 %) and CL3 batch lower (71.89 ± 0.906 %). The result reveals that as concentration of CS increases, process yield also increase and % loss of all batches very negligible during processing of freeze drying.

The % drug content of optimized CL4 batch was found to be 94.87 ± 0.495 %. The result reveals, low loss of drug content of optimized CL4 batch during freeze-drying.

The DSC thermograms studies show that the crystallinity of the drug has been reduced significantly in the nanoparticles. The result reveals that the prepared chitosan nanoparticles loaded lovastatin was present in the amorphous phase and may have been homogeneously dispersed in the chitosan matrix.

The diffraction spectrum of pure lovastatin showed that the drug was of crystalline nature as indicated by numerous, distinct peaks. The NP’s prepared with CS was characterized by less intensity of the diffraction peak, when compared to that of lovastatin.

The SEM studies revealed that lovastatin pure drug was a mixture of large crystals, indicating its crystalline nature. However, the prepared lovastatin loaded CS NP’s of batches were spherical shape and no drug crystals were present, which
was shown in SEM of pure lovastatin. The Transmission electron microscopy studies revealed that optimized batch CL4 was spherical shape and uniform size.

The % in vitro drug release after 60 min. dissolution studies, it was found that of pure drug tablet, marketed tablet and freeze dried optimized CL4 batch nanoparticles tablet, 28.51±0.243%, 43.82±0.113% and 85.12±0.374% respectively and best fit matrix model mechanism of CL4 batch.

In vivo hyperlipidemic activity performed on albino rats, after 21 days of treatment, results shows that plasma CH and TG levels were significantly lower (91.70± 0.047 mg/dl, 145.50± 0.037 mg/dl respectively) (p < 0.001) and HDL-CH levels were significantly higher (44.65 ± 0.02 mg/dl, p = 0.007) in TTG as compared to RTG. Thus, TTG showed a significantly better in vivo performance than RTG in terms of lipid profile parameters. (p < 0.001).

The pharmacokinetic parameters of lovastatin nanoparticles, lovastatin pure and lovastatin marketed tablets were compared in Albino rabbits. Cmax value for lovastatin nanoparticles, drug suspension and marketed tablet formulation was found to be 72.28 ± 0.158 ng/ml, 33.10 ± 0.176 ng/ml and 40.96 ± 0.244 ng/ml respectively, (p<0.001) indicating facilitated absorption of lovastatin by nanoparticles. T max of lovastatin nanoparticles was 2 hrs, whereas for the drug suspension and marketed tablet formulation was 1 h respectively. (p < 0.001) AUC (0 – 8 h) value for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 252.85 ± 0.134 (ng/ml × h), 65.86 ± 0.295 (ng/ml × h) and 107.56 ± 0.337 (ng/ml × h) respectively. (p < 0.001)

The results revealed that relative bioavailability was increased as compared to oral control group Standard I and II, it was found that relative bioavailability was 250.12 % and 407.99 % respectively. (p < 0.001) The in vivo study results reveal that lovastatin nanoparticles show better bioavailability than drug suspension and marketed formulation. The net result of drug entrapped in nanoparticles in nano range size that are absorbed lead to increase in bioavailability. The above results indicated that the lovastatin nanoparticles have the potential to be used to increase the oral bioavailability of highly lipophilic drugs.

Stability studies results indicate that after the 1, 2 and 3 months accelerated stability studies no morphological changes were observed but particle size increased, drug content and % cumulative release decreased in nanoparticles dispersion, freeze dried nanoparticles. After the 1, 2 and 3 months accelerated stability studies reveals % cumulative release decreased in pure tablet, marketed tablet and freeze dried nanoparticles tablets.

CONCLUSION

The present study was carried out to develop nanoparticles of simvastatin and lovastatin in order to enhance solubility, dissolution and bioavailability by decreasing the particle size of the drug. Successful incorporation of simvastatin
and lovastatin poorly soluble BCS class II drugs was carried out in to nanoparticles by precipitation-solvent displacement and ionotropic gelation technique. The precipitation-solvent displacement technique showed high entrapment efficiency (70.59-85.43%). The formulated simvastatin loaded nanoparticles exhibited nanometer size range spherical structure with fast release profile in vitro. The ionotropic gelation technique showed high entrapment efficiency (94.03-98.85%). The formulated lovastatin loaded nanoparticles exhibited nanometer size range spherical structure with fast release profile in vitro. Successfully entrapped a (lovastatin) hydrophobic molecule into hydrophilic nanoparticles formed by the process of ionotropic gelation method.

Successful conversion of dispersion in dry form can be carried out using freeze drying. The freeze-drying process produced nanoparticles in nanometer size. PXRD studies reveals decrease in crystallinity of nanoparticles. DSC studies result reveals that the prepared nanoparticles were present in the amorphous phase and may have been homogeneously dispersed in the polymer matrix. SEM & TEM studies prepared nanoparticles were spherical in shape and no drug crystals were present. The % in vitro drug of freeze dried optimized batches nanoparticles tablet, showed high drug release as compared to pure drug and marketed tablet respectively.

In vivo antihyperlipidemic studies on albino rats revealed that nanoparticles formulation showed a significantly better in vivo performance than reference formulation in terms of plasma lipid profile parameters. In vivo studies on rabbits revealed overall increase in bioavailability of the drug upon administration of nanoparticles formulation as compared with pure suspension and marketed formulation.

The experimental findings collectively support that prepared nanoparticles had the potential to enhance solubility, dissolution rate correlates with faster oral absorption and bioavailability of poorly water soluble drugs. Resulting in improved therapeutic outcome, thereby minimizing the dose-dependent adverse effects and maximizing the patients compliance, thus a promising drug delivery.

**FUTURE PROSPECTS**

Nanoparticles drug delivery system is gaining increased attention because of unique properties like their physicochemical diversity, biocompatibility, proved ability to encapsulate hydrophilic and lipophilic drugs; it can be used for enhancing bioavailability of the same.

Nanoparticles can be stored for longer period of time using freeze drying technique. The freeze dried nanoparticles can offer advantages of improved stability and dosing accuracy. They also offer for nanoparticles incorporation in to pellets, tablets and capsules. The experimental findings collectively support that nanoparticles is a potential carrier of hydrophilic and lipophilic drugs and thus a promising delivery system.
For the future, one could envision combining the concept of nano-sizing with other new approaches, such as tissue targeting and permeation enhancement. With better tools to deal with compound solubility, lead selection can be based on efficacy and safety alone. The preclinical and clinical trials need to be conducted on human study for the same formulation for conformation of efficacy and bioavailability. However, the fate of nanoparticles and mechanisms of enhanced absorption of water insoluble drugs need to be elucidated in future research.