Summary & Conclusions
SUMMARY AND CONCLUSIONS

The enhancement of oral bioavailability of poorly water soluble drugs which comes under BCS class II remains one of the most challenging aspects of drug development. Although there are many approaches available for improving dissolution rate and oral bioavailability, each method is having its own limitations. The present work focuses on the development of simvastatin loaded solid lipid nanoparticles using $3^2$ factorial design to enhance the oral bioavailability. Simvastatin is a poorly water soluble drug. It has short biological half-life of 3 hrs with bioavailability of less than 5%. The solid lipid nanoparticles were prepared using hot homogenization technique followed by ultrasonication method with three types of lipids i.e. tripalmitin, tristearin and trimyristin.

A validated HPLC method was developed for the in vitro and in vivo estimation of simvastatin in the prepared solid lipid nanoparticles. The steps involved in the extraction were reduced in this method. The method was found to be sensitive, precise, accurate and reproducible.

The solid lipid nanoparticles were developed by using $3^2$ factorial design. The concentrations of poloxamer 188 and soyalecithin were considered as independent variables. The particle size ($Y_1$), zeta potential ($Y_2$) and % entrapment efficiency ($Y_3$) were considered as dependent variables. The total numbers of runs required for carrying out the experiments were predicted by Design
Expert software trial version 8.0.7.1. The total number of runs predicted was found to be 9. The prepared solid lipid nanoparticles were characterized for physico-chemical parameters such as percent drug content, particle size distribution, % entrapment efficiency and zeta potential.

The concentration of poloxamer 188 and soylecithin which are independent variables produced predominant effects on mean particle size, entrapment efficiency and zeta potential values of the solid lipid nanoparticles. Each variable showed the independent effect as well as combined effect on the dosage forms. The increase in concentration of the poloxamer and soylecithin decreased the particle size and increased zeta potential and %entrapment efficiency.

The comparative values of $R^2$, adjusted $R^2$, predicted $R^2$, PRESS, s.d, %CV and significant P values ($p<0.05$) were calculated. The respective polynomial equations were derived.

All the three responses $Y_1$, $Y_2$, $Y_3$ for the prepared SLN were found to be independent for all the three bases used for their preparation. The three responses $Y_1$, $Y_2$ and $Y_3$ followed linear model for SLN prepared with trimyristin or tristearin. In case of tripalmitin based SLN, responses $Y_1$ and $Y_3$ followed linear model and $Y_2$ followed quadratic model.

The analysis of ANOVA data indicated the significance of models for all the three lipids used in the study. The desirability and counter plots were constructed and the optimized formulae were predicted by
using the constraints on the dependent variables. The constraints used in the study were zeta potential around 30 mV, maximum % entrapment (above 95%) and minimum particle size i.e. around 100 nm. Predicted model formulations were found matching with the optimized TMF9, TPF9 and TSF9 formulations. The % relative error was calculated between the experimental values and the predicted values of dependent variables. The maximum % relative error was found to be 1.3%. The values were found to be less than 5% and hence it confirmed the suitability of experimental design followed for this study.

Among all the formulations prepared with three bases of triglycerides TMF9, TPF9 and TSF9 formulations have shown the desired response. All the batches were prepared using 400 mg of triglyceride, 200 mg of poloxamer 188, and 200 mg of soya lecithin and 40 mg of simvastatin using 20 mL of dispersion medium.

The optimized batches were subjected for further evaluation parameters such as in vitro drug release studies, drug and excipients interaction studies by DSC, PXRD and stability studies. The in vitro drug diffusion studies using Franz diffusion cell indicated the slow release of drug compared to pure drug dispersion in 0.1% w/v sodium lauryl sulphate. Approximately 31-37% of drug was released in 36 hrs from the SLN compared to pure drug dispersion which released 98% in 36 hrs. These results are in agreement with earlier reported
studies. This confirmed the entrapment of drug in the lipid vehicles completely.

The *in vitro* drug release kinetics of SLN formulations indicated zero order with non-Fickian diffusion anomalous super case–II mechanism. The drug-excipient interaction studies carried out on the optimized formulations indicated no interaction between the drug and excipients used in the study.

The transmission electron microscope studies conducted on one of the optimized formulation TSF9 confirmed spherical shaped particles with complete entrapment of drug.

Among the optimized formulations using different triglycerides TSF9 gave the desired physico-chemical properties of particle size, zeta potential, %EE with higher drug diffusion of 37% in 36 hours and hence evaluated for *in vivo* studies.

The *in vivo* studies were carried out on male albino rats weighing around 200 to 250 g. The *in vivo* protocol was approved by Andhra university ethical committee (Regd.No.516/01/A/CPCSEA).

Approximately 2.07 fold increase of $C_{\text{max}}$ was observed for TSF9 SLN formulation when compared to pure simvastatin dispersion. The $t_{\text{max}}$ values were found to be in the range of 1 to 2 hr respectively for pure simvastatin dispersion and TSF9 formulation. The $t_{\text{max}}$ of the TSF9 formulation shifted by 1 hr from the $t_{\text{max}}$ of the pure simvastatin dispersion. The half-life increased by approximately 3.3 fold in TSF9 formulation compared to pure simvastatin dispersion.
3.3 fold and 4.0 fold increment in AUC$_{0-t}$ and AUC$_{0-\infty}$ was obtained in TSF9 formulation as compared to pure simvastatin dispersion. 7.55 fold and 11.03 fold increment in AUMC$_{0-t}$ and AUMC$_{0-\infty}$ was obtained in TSF9 SLN formulation as compared to pure simvastatin dispersion. 2.7 fold increments in MRT were obtained in TSF9 SLN formulation as compared to pure simvastatin dispersion.

The relative percent bioavailability (F$_{rel}$) of solid lipid nanoparticles (TSF9) was 406±61.29% indicating the enhanced oral bioavailability of solid lipid nanoparticles when compared to pure simvastatin dispersion.

Several factors have influenced the in vivo fate of the SLN after oral administration such as particle size, % entrapment efficiency, zeta potential, method of preparation, type of materials, their compositions and optimized process parameters for acceptable and stable SLN formation. In the present study the relative oral bioavailability of simvastatin from TSF9 formulation was significantly increased as compared to pure simvastatin dispersion because SLN are transported into systemic circulation via lymph up on oral administration. The lymphatic delivery is an alternative to avoid the first pass metabolism by oral delivery. The enhanced lymphatic transport of the drug reduced the hepatic first pass metabolism and improved relative bioavailability because intestinal lymph vessels drain directly into thoracic duct and further into the venous blood thus bypassing portal circulation. The main function of the lymphatic
system is to facilitate absorption of long chain fatty acids via chylomicron formation.

The SLN formulation could partially reduce the exposure of simvastatin to the CYP3A system in the intestinal gut during the absorption process which could result in the enhancement of $C_{\text{max}}$ and oral bioavailability of simvastatin.

In the present study SLN improved oral relative bioavailability of the simvastatin by virtue of their unique capability to bypass presystemic hepatic metabolism resulting in enhanced plasma concentration. The particle size of simvastatin was found to be around 100 nm, which might be the reason to enhance the rapid absorption of SLN as compared to pure simvastatin dispersion. These results suggested that solid lipid nanoparticles could be promising delivery systems to enhance the oral bioavailability of simvastatin.

The accelerated and long term stability studies were carried out for six months according to International Committee on Harmonization (ICH) guidelines to evaluate the stability of the optimized SLN formulation. At refrigerated conditions there were no major differences observed in the particle size, zeta potential entrapment efficiency and %drug content with respect to the initial values whereas changes were observed at accelerated conditions. In all cases, the %drug content and entrapment efficiency showed no much variation from initial data at long term condition compared to accelerated condition with all the different lipid bases used. Particle
size, PDI and zeta potential were found to be almost similar to the initial data in all the conditions loaded. Hence the recommended storage conditions for the prepared SLN could be under refrigerated conditions of 2-8°C. The outcome of the work clearly indicated the improvement of oral bioavailability of simvastatin, a BCS class II drug with its incorporation into SLN.

**Significant contributions of the present investigation**

1. For the first time, comparative studies with different triglycerides like trimyristin, tripalmitin and tristearin for improving bioavailability of BCS class II drug, simvastatin was carried out
2. $3^2$ factorial design was successfully used for the development of simvastatin loaded SLN.
3. The SLN enhanced the oral relative percent bioavailability of simvastatin.