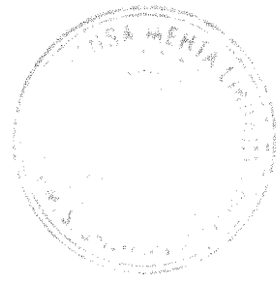

Chapter 10
Summary & Conclusion

SUMMARY AND CONCLUSION

The number of poorly soluble drugs is steadily increasing. About 40% of the drugs in the development pipe lines and approximately 60% of the drugs coming directly from chemical synthesis are poorly soluble. However, there are also drugs which show low permeability that can be caused by the efflux pumps present in the gastrointestinal drugs, e.g. p-glycoprotein. Consequently most of them exhibit a poor oral bioavailability because in general low solubility and low permeability is correlated with poor oral bioavailability. The currently available conventional dosage forms are mostly immediate release, require frequent dosing and are associated with non – compliance. The challenge is to create novel drug delivery systems which can overcome these drawbacks associated with conventional therapy. The proposed novel delivery system should not only dissolve drug fast but should be combined with a technology to improve bioavailability.

In the present vocation, we propose Nanoparticulate delivery systems for Saquinavir and Adefovir Dipivoxil for improvement of oral bioavailability.

It is envisaged that the proposed delivery systems will improve solubility and permeability of drug which will result in increased bioavailability. It is expected to provide sustained release of drugs which will reduce the frequency of administration of conventional dosage form and will improve patient compliance. This thesis work explains the development, characterization and in vivo evaluation of Solid lipid Nanoparticles and Nanosuspensions of Saquinavir and Adefovir Dipivoxil.

Saquinavir (SQ)

Solid lipid Nanoparticles (SLN)

Partition coefficient study was carried out prior to preparation of SLN. Solid lipids such as Tristearin, Glyceryl Monostearate, Glyceryl behenate, Stearic acid, Witepsol E 85 were studied. Saquinavir showed higher partition values in Stearic acid (3.42 ± 0.15) and Glyceryl Monostearate (3.09 ± 0.11), hence they were selected for further study. Preparation of Saquinavir SLN (SQSLN) was carried out by High Pressure Homogenization method. Formulation development was carried out by optimizing process and formulation parameters. Prior to the formulation step, the possible process parameter influencing the formation of nanoparticles, size and polydispersity

of nanoparticles was identified and optimized. The parameter studied was number of homogenization cycles. Optimum number of homogenization cycles resulting in smaller particle size with narrow size distribution was 3 cycles for both GMS and SA SLN. Formulation parameters such as Type of emulsifier, Concentration of emulsifier and concentration of Saquinavir (w.r.t.lipid) were optimized. Optimization of emulsifier was carried out using Poloxamer 407, Tween 80 and combination of Poloxamer 407: Tween 80 at different ratios (1:1, 1:3 and 1:5). It was found that combination of Poloxamer 407: Tween 80 at 1:3 ratio showed minimum particle size for GMS SLN (215 ± 9 nm) and SA SLN (180 ± 10 nm). The optimum concentration of Poloxamer 407: Tween 80 ratio needed to produce smallest particle size (194 ± 12 nm) of SA SLNs was 3% w/v while that of GMS SLNs was 4% w/v (237 ± 21 nm). The concentration of SQ was varied between 0.5 %, 1 % and 2 %w/v while keeping the lipid concentration constant at 5 % w/v. Entrapment efficiency was found to be more in case of SA SLN (79.24 % ± 1.53)compared to GMS SLN (72 % ± 1.23).

Optimized formulation was characterized by Differential Scanning Calorimetry (DSC), X Ray diffraction study (XRD), Transmission Electron microscopy (TEM) and *in vitro* release study. DSC study of SQ loaded SA SLN and SQ loaded GMS SLN showed reduction in melting point of lipid which may be due to increased number of lattice defects in lipid. In XRD spectra of SQSLN, reduction in the intensity of characteristic peaks of SQ was observed which may be attributed to the incorporation of SQ in between the crystal lattice of the lipid leading to decrease in the crystallinity of SLN. TEM image revealed spherical shape particles with smooth and nonporous surface. pH of the medium was found to have drastic influence on the in vitro release of the drug. The release rate was found to be in the decreasing order of 0.1 N HCl < pH 4.5 < pH 7.2. After 24 hrs, 96.69 ± 4.51 % of SQ released from SGMLN while 76.2 ± 4.5 % of SQ released from SQSLN. The release profiles were then fitted into different exponential equations such as zero order, first order, Higuchi and Peppas-Korsemeyer and best fit was determined. All the kinetic models, other than the first order, fitted well. The goodness of fit for various models investigated for SQSLN and SGMLN ranked in the order of Higuchi ≈ Peppas > zero order > first-order.

Short-term stability study of SQSLN dispersion at Room Temperature (RT) for 15 days showed increase in particle size upto 1.091 ± 27 nm (PDI – 0.391) while Zeta

potential reduced from -30.21 mv to -9.02 mv. It indicates that prepared SLNs dispersion was not stable enough at R.T. Hence, further stability study was carried out by keeping SLN dispersion at 2-8 ^{0}C while lyophilized SLNs were kept at 2-8 ^{0}C and RT for 3 months. It was found that lyophilized SLNs were more stable than SLN dispersion at both 2 – 8 ^{0}C and room temperature. GI stability study was carried out by incubating SLN dispersion in 0.1 N HCl for 2 hours. Saquinavir loaded SLN formulation found to be unstable in terms of particle size & zeta potential after 2 hrs of incubation although formation of microparticles was not observed.

Nanosuspension:

Saquinavir Nanosuspension (SNS) was prepared by Pearl milling technique using Zirconium oxide beads. Optimization study was carried out to assess effect of process and formulation parameters on particle size of nanosuspension. The parameters studied were milling time, ratio of beads and selection of surfactant. Minimum particle size was obtained after 10 hours of milling (0.356 ± 0.010 µm). Ratio of bead was selected as 75 : 25 (small :large). During the course of optimization, the type of surfactant was chosen between Poloxamer 407, Tween 80, PVP K30 and Poloxamer 407: Tween 80. Formulation prepared with Tween 80 showed smallest particle diameter (429 ± 14 nm) compared to other surfactants. Further optimization was carried out by 3^2 Factorial Design. The Volume of milling media **(X_1)** and concentration of surfactant **(X_2)** were studied for their effect on Mean particle diameter - Day 0 **(Y_1)** and Mean particle diameter - Day 7 **(Y_2)**. On analyzing the data of all the 9 formulations prepared as per 3^2 Factorial design using Design Expert® software, various polynomial equations, response surface and contour plots were generated. The effect of volume of milling media seems to be more pronounced as compared with that of concentration of surfactant on Mean particle diameter - Day 0 while effect of concentration of surfactant on Mean Particle diameter - Day 7 is more pronounced than effect of volume of milling media. The optimized formula was arrived by keeping the Mean particle diameter – Day 0 in range of 300 to 400 nm. Another dependant variable Mean particle diameter – Day 7 was kept at minimum level. Formulation SQ9 (containing high (+1) levels of variables, X_1 and X_2) fulfilled all the criteria set from desirability search. DSC study of SNS showed reduction in melting point of SQ and broadening of SQ peak which indicated that SQ might be

converted to an amorphous state. In case of XRD of SNS, reduction in intensity of peak was observed at characteristic 2θ angle values of SQ. This may be due to small particle size (nanometer range), high specific surface area and presence of surfactant in nanosuspension. TEM image reveals that particles are discrete and non aggregated and are almost spherical in shape. SQ from SNS showed 96.66 ± 1.98 % release in 20 minutes in pH 7.2 buffer while SQ from SMS showed 97.21 ± 1.12 % in about 90 minutes. Increase in release rate can be attributed to increase in the surface area after nanosizing the crystals. The release profiles were then fitted into different exponential equations such as zero order, first order, higuchi, and Peppas- Korsmeyer to characterize the release. It was found that drug release from SMS followed Peppas – Korsmeyer (r^2=0.860) while release of SNS was found to follow Higuchi (r^2=0.989).Value of *'n'* indicates that SMS followed Super case II transport while SNS followed Case II transport.

Pharmacokinetic study of Saquinavir containing nanoparticulate delivery systems (SLN and Nanosuspension) in blood displayed an increase in AUC and hence relative bioavailability when compared with oral administration of conventional suspension (SMS). The relative bioavailability for SQSLN and SLN were 37.39 % and 66.53% respectively compared to 18.87 % by oral administration of SMS. It has indicated improvement in bioavailability of SQ when given as SQSLN and SNS. Highest C_{max} (6.43± 0.47 µg/gm) amongst all tested formulations was observed with SQSLN followed by SNS (5.72 ± 0.57) and SMS (2.72 ± 0.67) . The sustained-release characteristic of the SQSLN was reflected in the MRT in the body. MRT was considerably increased following administration of the SQSLN as compared to SNS. The average MRT after oral administration of SQSLN, SNS and SMS were 23.82 ± 0.90, 15.42 ± 0.57 and 17.51 ± 0.76 h, respectively, as compared to 10.41 ± 0.62 after I.V. administration

Adefovir Dipivoxil (ADF)

Solid Lipid Nanoparticles (SLN)

Partition coefficient study of Adefovir dipivoxil was carried out in different lipids (Glyceryl monostearate, Glyceryl palmitostearate, Tristearin, Trimyristin, Stearic acid and Cetyl palmitate). It has shown highest partition coefficient value of 2.48 in

Trimyristin. Adefovir loaded SLNs (ADSLN) were prepared by solvent injection method. The possible parameters influencing the formation of nanoparticles and their size and polydispersity were identified and optimized. Process parameters like stirring speed and stirring time and formulation parameters like organic solvent, lipid and drug loading, selection of surfactant and concentration of surfactant were optimized. Suitable stirring conditions were found to be stirring speed at 600 rpm for 20 mins. Different organic solvents (Acetone, IPA and DCM : IPA) were studied at 4, 6 and 10 ml volume. It was found that 6 ml of IPA showed minimum particle size (260 ± 15 nm). Lipid loading was studied at 0.5 %, 1% and 1.5 % w/v. At 0.5%w/v concentration of the lipid and 6ml of IPA, aggregates were not formed and mean particle diameter was 255 ± 21 nm with PI of 0.212. Effect of surfactants (Soya PC, Poloxamer-407, Transcutol P and Soya PC : Poloxamer 407) on particle size, size distribution, and zeta potential was studied. The minimum particle diameter (267 ± 18 nm) and highest zeta values (-26.9 mv) were observed with Soya PC: Poloxamer 407 at 1:4 ratios. Effect of drug loading (2%, 4%, 6% and 8 % w/w) with respect to total lipid content was examined. The results indicate increase in drug loading from 2% w/w to 8% w/w resulted in decrease in entrapment from 73.5 ± 2.12 % to 31.15 ± 3.35 %. So the optimum drug loading was chosen at 2 % w/w of lipid content. In case of DSC curve of ADSLN, absence of ADF peak near its M.P. indicates that drug might have entrapped in lipid matrix. In case of XRD study, characteristic peak of lipid did not shift. However, there was a slight reduction in the intensity of the peaks which may be attributed to the incorporation of ADF in between the crystal lattice of the lipid leading to decrease in the crystallinity of ADF loaded SLN. TEM image revealed round shape with smooth surface. Although, some of the particles are found to be of irregular shape. In vitro release of ADSLN was found to be faster in the case of acidic media (pH 2) compared to pH 4.5 buffer and pH 7.2 buffer. It was found that drug release in all media followed Peppas – Korsmeyer model more than Higuchi, Zero order and First order.

SLN dispersion kept at room temp. for 15 days showed increase in particle size from 260 ± 21 nm to 982 ± 22 nm (PDI – 0.442) while Zeta potential reduced from -26.6 mv to -10.22 mv. It indicates that prepared SLNs dispersion was not stable enough at RT. Hence long term stability study was performed by keeping SLN dispersion at 2-8 ^{0}C while lyophilized SLN at 2-8 ^{0}C and RT for 3 months. Lyophilized SLNs were

found to be more stable than dispersion at both 2 – 8 ^{0}C and room temperature. Incubation of ADSLN dispersion in 0.1 N HCl for 2 hours showed increase in particle size and reduction in zeta potential.

Nanosuspension:

Pearl milling technique was used for preparation of Adefovir dipivoxil Nanosuspension (ANS). Prelimnary optimization study was carried out to assess effect of process parameters (milling time, ratio of beads) and formulation (selection of surfactant) on particle size of nanosuspension. The Mean particle diameter of bulk ADF was 710.05 ± 70 µm. Pearl milling of 18 h resulted in particles with mean particle diameter of 0.422 ± 0.026µm (PI – 0.343). Minimum particle size i.e. 434 ± 17 nm was obtained when 50:50 ratio of small (0.4 mm - 0.7 mm) : large (1.2 mm - 1.5 mm) size range beads were used.

The type of surfactant was selected from Poloxamer 407, Sodium cholate Tween 80, and Poloxamer 407 : Sodium cholate. Formulation prepared with Tween 80 showed smallest particle diameter (429 ± 14 nm) compared to other surfactants. Further optimization was carried out by 3^2 Factorial Design. The Volume of milling media (X_1) and concentration of surfactant (X_2) were studied for their effect on Mean particle diameter - Day 0 (Y_1) and Mean particle diameter - Day 7 (Y_2). On analyzing the data of all the 9 formulations prepared as per 3^2 Factorial design using Design Expert® software, various polynomial equations, response surface and contour plots were generated. The effect of concentration of surfactant seems to be more pronounced as compared with that of volume of milling media on Mean particle diameter - Day 0. In case of Mean Particle diameter - Day 7, effect of volume of milling media was more pronounced than effect of concentration of surfactant. The optimized formula was arrived by keeping the Mean particle diameter – Day 0 in range of 300 to 400 nm and Mean particle diameter – Day 7 in the range of 400 to 450 nm. In DSC study of ADSLN, peak of ADF was found to be broader which is attributed reduced degree of crystallinity as a result of pearl milling. TEM image of ANS revealed particles as homogenously distributed and not as aggregates of nanoparticle

In case of XRD of ANS, reduction in intensity of peak & peak broadening was observed at characteristic 2θ angle values of ADF. This may be due to small particle size (nanometer range), high specific surface area and presence of surfactant in

nanosuspension. In order to assess whether the goal of improving the dissolution rate of ADF from nanosuspension was achieved, in vitro dissolution profiles of ADF from suspension and nanosuspension were compared. The release profiles clearly indicated the faster dissolution rate of ADF from nanosuspension (97.36 ± 1.71 % in 5 minutes) compared to suspension (96.66 ± 1.33 % in 25 minutes). The release profiles were then fitted into different exponential equations such as zero order, first order, higuchi, and Peppas Korsemeyer to characterize the release. It was found that drug release in AMS and ANS followed by Peppas Korsemeyer model more than Higuchi, Zero order and First order.

Plasma concentration – time profile of Adefovir containing nanoparticulate delivery systems (SLN and Nanosuspension) displayed an increase in AUC and hence relative bioavailability when compared with oral administration of conventional Adefovir dipivoxil suspension (AMS). The relative bioavailability for ANS and ADSLN were 66.36 % and 78.56 % respectively compared to 41.68 % bioavailability obtained after administration of AMS. It indicates improvement in bioavailability of Adefovir from nanoparticulate formulation than conventional suspension. Highest C_{max} (252±15.01µg/gm) amongst all tested formulations was observed with ANS followed by ADSLN (176±5.77 µg/gm) and AMS (252±15.01 µg/gm) .The sustained-release characteristic of the ADSLN was reflected in the MRT in the body. MRT was considerably increased following administration of the ADSLN as compared to ANS. The average MRT after oral administration of ADSLN, ANS and AMS were 19.74± 1.24, 9.412± 2.01 and 10.45± 1.52 h, respectively, as compared to 4.05±1.34 h.

Biodistribution of Adefovir Dipivoxil was studied following oral administration. Three formulations were tested: a nanosuspension (ANS), one solid lipid nanoparticle preparation (ADSLN) and one suspension in micron size (AMS). Both colloidal drug delivery systems (ANS and ADSLN) have shown increase in bioavailability compared to conventional suspension or microsuspension (AMS). Amongst colloidal drug delivery systems, SLN showed prolonged residence time in blood (MRT = 19.74 ± 1.24) compared to ANS (MRT=9.41 ± 2.01). Significant difference was observed in Cmax and AUC (0 → inf) between ANS, ADSLN compared to AMS.

Conclusion

From literature review, two antiviral drugs (SQ and ADF) are selected based on the low solubility and low bioavailability. Nanoparticulate drug delivery systems (SLN and NS) were prepared for these drugs. Enhanced dissolution was observed with these systems compared to conventional suspension. Also, sustained release was observed in case of SLN formulation. In the *in vivo* study, the relative bioavailability of Saquinavir SLN and Nanosuspension formulation was 66.53% and 37.53 % compared to relative bioavailability of conventional suspension i.e. 18.87%. Similarly, the relative bioavailability of Adefovir dipivoxil SLN and Nanosuspension formulation was 78.56% and 66.36 % compared to relative bioavailability of conventional suspension i.e. 41.68%.

The results of the present investigations conclusively indicate enhancement in bioavailability of the drugs, Saquinavir and Adefovir Dipivoxil when administered as Solid lipid nanoparticles and Nanosuspension. Moreover, sustained release and improvement in bioavailability from SLN formulation would lead to reduction in dosage and dosing frequency of these drugs. This in turn reduces cost of the therapy for treatment of viral infection and also improves patient compliance. Hence, the developed nanoparticulate delivery systems of Saquinavir and Adefovir Dipivoxil hold promise as better alternative to the conventional formulations. This study presents some new findings, which may be exploited in improving the therapeutic efficacy of drugs by formulating in Nanoparticulate drug delivery systems.

Further investigations in human beings under clinical conditions are necessary before these systems can be fully exploited.