CHAPTER VII

SUMMARY

Zidovudine (AZT) the first anti-HIV compound approved for clinical use is still widely used alone or in combination with other antiviral agents for treatment of AIDS and AIDS-related complex. The main limitations on the therapeutic effectiveness of AZT are its dose-dependent hematological toxicity, high first-pass metabolism, poor bioavailability and very short biological half-life (Kieburtz et al., 1992). After oral administration it is rapidly absorbed from the gastrointestinal tract with a peak plasma concentration of 1.2µg/ml, at 0.8 h. It is also rapidly metabolized to the inactive glucuronide with a mean elimination half-life \( t_{1/2} \) of 1 h. This necessitates frequent administration of large doses (200 mg every 4 h), since it is crucial to maintain the systemic drug concentration within the therapeutic level throughout the treatment course.

HIV primarily infects helper T cells, macrophages and dendritic cells, which are vital to the human immune system. Macrophages are stationed at strategic points like liver, spleen, lungs and connective tissues where microbial invasions are likely to occur (Julie et al., 2008) and becomes a reservoir for HIV and viral replication occurs in the macrophages after infection (Arun et al., 2000).

The research work entitled "Formulation Development and in vivo Evaluation of Zidovudine Niosomes" was undertaken with the following objective.

The objective of this study was to evaluate the process parameters necessary to prepare niosome vesicles in the size range of 100-1000 nm for improving entrapment, release, tissue distribution of AZT to macrophages which acts as reservoir for HIV virus. Targeting will improve the availability of the drug at the site, which will reduce the dose and decrease the risk of adverse side effects.
The study has been presented in 7 chapters with introduction in chapter 1, followed by literature review in chapter 2. The Drug and polymer profile was discussed in chapter 3. The objectives of the study were briefed in chapter 4 and elaborate methodology in chapter 5. Results and discussion was made in chapter 6. Summary was presented in chapter 7. Conclusion was finally presented in chapter 8 with up to date bibliography till 2009 related to the study at the end.

Multilamellar niosomes were prepared by thin-film hydration method. The process-related variables such as sonication time, hydration medium, hydration time, speed of rotation of flask evaporator and charge-inducing agents were investigated in vesicle formation with 90 µM Tween 80 and 20 µM cholesterol with a fixed amount of zidovudine. From this studies, formulation with a Tween 80:cholesterol ratio of 1:4.5 µmol, 10 ml of chloroform as solvent, 45 min hydration time with phosphate buffered saline at pH 7.4, 100 rpm of the evaporator flask, 5 µM charge-inducing agent, and 2 min of sonication showed maximum drug entrapment of 80.3 %. Hence, these parameters were used to prepare other formulations with different non-ionic surfactants for further studies.

The size, shape and lamellar nature of vesicles in non sonicated formulations were observed by optical microscopy using a calibrated eyepiece micrometer. Non-sonicated vesicles were in the size range of 2 to 5µ. Tween and Span micelles without cholesterol were larger in size than vesicles with cholesterol.

Sonicated vesicles were in the submicron size range. Scanning electron microscope study was avoided because during drying surfactants present in niosomes deposits on the surface. TEM images of niosomes showed that the sonicated Tween 80 vesicles with and without DCP were in nanosize with mean diameter of 149.62 and 137.28 nm respectively. Sonicated vesicles without DCP had a narrower size distribution (polydispersity index) and were smaller than those with DCP. Tween 80 niosomes possess a negative charge of -0.0925 mV at pH 7.4, indicating that a weak electrostatic repulsive force exists in the
niosomal bilayer. Also, the inclusion of DCP in Tween 80 niosomes increased the zeta-potential (-7.79 mV) as compared to the Tween 80 formulation without DCP.

Formulations without cholesterol (Span 20 – 4.03 cps, Span 40 – 4.16 cps) have a higher viscosity than formulations with cholesterol (Span 20 – 3.24 cps, Span 40 – 2.13 cps) and formulations with cholesterol and DCP (Span 20 – 3.48 cps, Span 40 – 3.42 cps).

Osmotic shock study results reveal that vesicle size was not increased significantly in formulations with cholesterol when compared with formulations without cholesterol. Formulations incubated with saline showed a slight increase in vesicle size which confirms zidovudine niosomes could be diluted with normal saline for parenteral use.

Micelles prepared without cholesterol entrap low amounts of drug in Tween and Span formulations. Increase in surfactant beyond a ratio of cholesterol: surfactant of 1:4.5 results in spherical vesicles along with aggregates. Entrapment efficiency for niosomes prepared with Tween 20 was higher than that with Tween 60. Similarly, the entrapment efficiency of Tween 60 was higher than Tween 40 and Tween 80. The entrapment efficiency of Span 60 was higher than Span 20, 40 and 80.

Increase in entrapment was observed after the inclusion of charge inducing agent in Tween 80, Span 20, 80 formulations. Similarly, decrease in entrapment was observed in Tween 20, 40, 60 and Span 40, 60 formulations.

Niosomal zidovudine formulations with Tween 20, 40, 60 and 80 showed significant reduction in in vitro drug release (p<0.001) for 4 h when compared with drug in solution. When the concentration of cholesterol was high in Tween 80 (cholesterol: surfactant μmolar ratio 1:1.5) the drug release was 41.73 % in 3 h, and in formulations with a low concentration of cholesterol (cholesterol:surfactant μmolar ratio 1:6), the percentage drug release was 70.16 %. The release from Tween 80 formulations (cholesterol:surfactant μmolar ratio 1:4.5) was 78% and from Tween 60 formulations, 82% in 12 h. Drug release
from Span 80, 60, 40 and 20 (cholesterol:surfactant molar ratio 1:4.5) was found to be 66.8%, 59.9%, 62.5% and 75.1% in 9 h. Inclusion of DCP in Tween and Span formulations increased the percent release of drug and extended the time of release compared with formulations without DCP.

Tween formulations without cholesterol released the drug within 6 to 8 h. Release from Span 40 and Span 60 formulation was 69.59% and 72.08% up to 11 h in the absence of cholesterol; but span 20 and span 80 showed fast release in 5 hours of 59.4% and 61.09% respectively.

Tween 20 with cholesterol follow zero-order kinetics and the other formulations obey first-order kinetics. Higuchi’s correlation coefficient confirms that drug release was proportional to the square root of time indicating that zidovudine release from niosomes was diffusion controlled.

High proportion of drug was distributed in liver and spleen of mice after single IV bolus injection of AZT niosomes when compared with drug in solution. In Tween 80 formulation with DCP, the drug concentration in liver and spleen were reduced as compared to Tween 80 formulation without DCP. In Span 20 formulation with and without DCP, drug distribution was not found in spleen. Plasma concentration was found to be high for drug in solution when compared to niosomal formulation.

AZT in solution was rapidly eliminated after the intravenous dose. Clearance of AZT from plasma was slower for Tween 80 formulation with and without DCP as compared to drug in solution. Significant increase (P<0.001) in the AUC\textsubscript{0→last}, MRT and V\textsubscript{d} was observed in niosome formulation with and without DCP as compared to free drug solution. Pharmacokinetic data confirm that the maximum bioavailability could be achieved by administering a minimum dose of drug in niosomal form. Relative bioavailability was found to be high for Tween 80 niosomes formulated with DCP when compared to Tween 80 niosomes without DCP.
In stability study, no change in colour, turbidity and pH were observed during 90 days of storage at a room temperature of 28 ± 2°C and at a relative humidity of 65 ± 5 %. Niosomal formulations stored at refrigerated condition (2–8°C) showed a drug leakage of 4 – 6.5 %, whereas formulations stored at an accelerated condition of 28 ± 2°C showed an increased drug leakage of 18 - 25 % after 90 days. Proniosomes overcomes the drawback of freeze-dried niosomal formulations where exposure of the product to room temperature converts it into a liquid. Vesicles were observed in the reconstituted proniosomes that were stored for 3 months at 28 ± 2 °C.

Tissue distribution study of Tween 80 proniosomes revealed that the drug concentration was found only in plasma and kidney and in specific a higher concentration was found in kidney. This confirms that conversion of niosomal formulation to proniosomes alters the uptake of vesicles by RES which results in poor distribution to organs.

Rapid clearance was found in Tween 80 proniosomes. Decrease in AUC, AUMC, half-life and MRT were found for proniosomes when compared with niosome formulation. Relative bioavailability was found to be low for Tween 80 proniosomes when compared to Tween 80 niosomes.

Drug content was determined immediately after converting niosomes to proniosomes and it was found to be 79.1 % and for the formulation stored for 90 days at room temperature it was found to be 76.9%. This result reveals that conversion of niosomes to proniosomes decreases the leakage of drug from vesicles during storage and thus increases the stability.