CHAPTER 9

SUMMARY AND CONCLUSIONS

SUMMARY:

The present study involves the preparation of Niosomes containing two different drugs namely Rifampicin and Gentamicin sulphate, separately, which could be used to achieve natural or passive targeting.

These niosomal drug delivery systems include.

1. Niosomes of Rifampicin prepared using Span and Tween series of surfactants.

2. Niosomes of Gentamicin sulphate prepared using Span and Tween surfactants.

These systems were optimized with respect to their formulation and method of preparation. They were characterized with respect to drug entrapment efficiency, particle size analysis, surface polarity, morphological characteristics and Invitro drug leakage rate study.

Selected niosomes were also investigated for pharmacokinetic parameter and invivo organ distribution pattern in organs like liver, lung, kidney, and blood serum following intravenous administration and intratracheal administration in rat model.

Niosomes containing Rifampicin:

Niosomes containing Rifampicin were prepared using a series of surfactants of Span series namely Span 40, Span 20, Span 60 and Span 80 and Tween surfactants namely Tween 20, Tween 40, Tween 60 and Tween 80 by the thin film hydration method[50].

Niosomes were separated from unentrapped drug by gel filtration technique (Mini column centrifugation method) through sephadex G-50 column.

Cholestrol was incorporated in niosome formulation. Drug entrapment efficiency was determined with and without admixture of cholesterol It was found that inclusion of cholesterol increased the entrapment efficiency of drug in niosomes

Among the batches of niosomes prepared, those prepared using Tween series of surfactants, gave very poor entrapment of drug.

The drug entrapment efficiency of various formulations were compared. Based on this data, it was found that niosomes prepared with Span 60 gave maximum drug entrapment. Hence niosomes prepared from Span 60 were selected for detailed studies.

Process variables like solvent, sonication time, volume of hydrating medium and hydration time were optimized to prepare niosomes containing Rifampicin. Influence of the process variables of the Rifampicin niosomes on the entrapment efficiency was studied by 2^4 factorial design (Yate's treatment)[152].

The size analysis and size distribution of niosomes which were prepared using series of Span surfactants, were determined using optical microscope.

Vesicle size of all formulations of Niosomes were compared. The mean size of vesicles in Span 60 niosomes was found to be 7.5μm.

Photomicrograph of Niosomes were obtained with Olympus 201 microscope and scanning electron microscope and they indicated a spherical multilamellar vesicles with a distinct boundary.

Niosomes were evaluated for stability with respect to drug leakage. Niosomes prepared from Span 60 and cholesterol were stored at different temperatures like refrigeration temperature (5°C) and ambient temperature (25°C to 35°C) in the form of aqueous suspension.

The extent of drug leakage from these niosomes was determined upto a period of 60 days after removing aliquots at various time intervals. The aliquots were gel filtered and amount of drug leakage was determined by analyzing the eluent.

At 5°C the efflux of Rifampicin from niosomes prepared by thin film hydration method, exhibit biphasic drug release profile, an initial slow release phase from first day to 40^{th} day. This may be due to the presence of saturated periferal layer in the beginning followed by the diffusion of drug through the lipid layers.

At ambient temperature the efflux of Rifampicin from niosomes exhibited monophasic drug release pattern. The rate of drug leakage was determined by monophasic equation.

Invitro affinity studies have been conducted to investigate the interaction of macrophages and niosomes.

Invivo organ distribution pattern of niosomes was determined by intravenous administration and intratracheal administration of niosomes in healthy albino rates (wistar strain). The animals were sacrificed at the intervals of 1,3,6,12 and 24 hours and organs like the lung, liver and kidney were removed and homogenized. The amount of Rifampicin in the organ homogenate was estimated by the high performance liquid chromatography method after the extraction of drug from the homogenate. The drug content in the blood serum was also estimated,

and pharmacokinetic parameters were determined. Compared to a free drug solution, niosomes following intravenous administration showed higher value of area under the curve (AUC) for lungs (46824.25) and less value of AUC for liver (66.83), kidney (1227.3) and serum (0.4257), while intratracheal administration of niosomes showed high value of AUC for lungs (51353.65) and less value of serum (0.4228).

Efficacy of intratracheal administration of niosomal suspension of Rifampicin was evaluated in terms of ratio of AUC for drug concentration in lungs and serum following intratracheal and intravenous administration. These values were found to be 1.09673 for lungs and 0.9932 for serum. This indicates the marginally higher availability of drug in lungs following intratracheal administration in comparison Intravenous administration. Serum level was found unchanged.

The invivo organ distribution studies with Rifampicin niosomes showed a slow drug release behavior with mean residence time (MRT) value for lung following intravenous administration to be and it was 10 015 hours and 12.30 hours in case of intratracheal administration. Compared to free drug solution, niosomes showed the concentration of 950.80μg/g in lung 1.073μg/g in liver, 13.32 μg/g in kidney and 0.0012μg/ml in serum at 24th hour after intravenous administration. After intratracheal administration, concentration was found to be 1772.09 μg/g in lung and 0.0041 μg/ml in serum at 24th hour.

The tissue distribution of Rifampicin Niosome was examined after intravenous route and intratracheal administration in rats and the results were compared with the plain drug solution. The niosomes encapsulated form showed significantly higher accumulation in the lung as compared with plain drug solution, in both route of administration The results were subjected to Analysis of variance to determine the level of significance. ANOVA showed that highest value of significance (F=23.38) for drug accumulation in the lung from niosomal when compared with the plain Rifampicin administered by both routes.

Niosomes containing Gentamicin sulphate:

Niosomes containing Gentamicin sulphate were prepared using a series of Tween surfactants namely Tween 20, Tween 40, Tween 60, and Tween 80 and Span surfactants namely Span 20, Span 40, Span 60 and Span 80 by thin film hydration method.

Niosomes were separated from unentrapped drug by dialyzing exhaustively in dialysis tubing, against distilled water.

Cholesterol was incorporated in niosome formulation. Drug entrapment efficiency was determined with and without admixture of cholesterol. It was found that inclusion of cholesterol increased the entrapment efficiency of the drug in niosomes.

Gentamicin niosomes prepared with Span series of surfactants gave very poor drug entrapment when compared with the Tweens The

drug entrapment efficiency of various formulations were compared. Based on this data, it was found that niosomes prepared with Tween 60 gave maximum drug entrapment. Hence niosomes prepared from Tween 60 were used for further studies.

Process variables like solvent, sonication time, volume of hydrating medium and hydration time were optimized to prepare niosomes containing gentamicin sulphate. Influence of the process variables of the gentamicin sulphate niosomes on the entrapment efficiency was studied by 2^4 factorial design (Yate's treatment).

The size analysis and size distributions of niosomes which were prepared using series of Tween surfactants, were determined using optical microscope.

Vesicle size of all formulations of niosomes were compared. The mean size of vesicles in Tween 60 niosomes was found to be 7.4μm.

Photomicrograph of niosomes were obtained with Olympus 201 microscope and scanning electron microscope and they indicated a spherical multilamellar vesicles with a distinct boundary.

Niosomes were evaluated for the stability with respect to drug leakage. Niosomes prepared using Tween 60 and cholesterol were stored in aqueous suspension form at two different temperatures, viz.

refrigeration temperature (5°C) and ambient temperature (25°C to 35°C). The extent of the drug leakage from these niosomes was determined by analyzing the eluent.

At 5°C the efflux of gentamicin sulphate from niosomes prepared by the thin film hydration method, exhibited biphasic drug release profile, an initial slow release phase from 1st day to 32nd day. This may be due to the presence of saturated layer in the beginning followed by the diffusion of drug through the lipid layers.

At ambient temperature the efflux of gentamicin from niosomes exhibited monophasic drug release pattern. The rate of drug leakage was determined by monophasic equation.

Invivo organ distribution pattern of niosomes was determined by intravenous administration and intratracheal administration ofniosomes in healthy albino rats (wistar strain). The animals were sacrificed at the intervals of 1,3,6,12 and 24 hours and organs like lung, liver and kidney were removed and homogenized. The amount of gentamicin in the organ homogenate was estimated by UV-visible spectrophotometric method after the extraction of drug from the homogenate. The drug content in the blood serum was also estimated, and pharmacokinetic parameters were determined. Compared to the free drug solution,

niosomes following the intravenous administration showed higher value of area under the curve (AUC) for lungs (14787.135) and less value of AUC for liver (361.155), kidney (1259.29) and serum (70.80) while intratracheal administration of niosomes showed high value of AUC for lungs (17504.49) and less value for serum (36.19).

Efficacy of intratracheal administration of niosomal suspension of Gentamicin was evaluated in terms of ratio of AUC for drug concentration in lungs and serum.

Following intratracheal administration values were found to be 1 18376 for lungs and 0.5112 for serum. This indicates the high availability of drug in lungs and low availability of drug in serum.

The invivo organ distribution studies with gentamicin niosomes showed a slow drug release behaviour with Mean Residence Rime (MRT) value for lung following intravenous administration was 10.61 hours and 13.24 hours in case of intratracheal administration. Compared to free drug solution, niosomes showed the concentration of 485.05μg/g in lung, 5.94μg/g in liver, 10.68μg/g in kidney, 1.7μg/ml in serum at 24th hour. A significant difference in the total drug concentration in the lung, liver, kidney and blood serum was found between niosomes and free gentamicin solution ($p<0.001$). Out of the four organs, significant accumulation of gentamicin was found in the lung following niosome administration as compared to the drug solution. The results

were subjected to Analysis of variance to determine the level of significance. ANOVA showed that highest value of significance (F=17.65) for drug accumulation in the lung from niosomes when compared with plain gentamicin administered by both the routes.

Conclusion:

Rifampicin is the first line drug used in the treatment of tuberculosis and demands a high dose drug treatment over a period of 4-6 months. The causative organism is known to develop resistance if drug blood levels remain below the minimum effective concentration leading to the clinical failure Rifampicin also has various side effects.

Gentamicin has been used as a standard therapy for Nosocomial pneumonia during the past 20 years and it exhibits a concentration dependent activity Nosocomial pneumonia occurs in 0 6-1 of patients admitted to a gental hospital. It is frequently caused by Gram -negative bacilli and mortality rates of 20-50 percent have been reported. So appropriate antimicrobial therapy is the most important factor determining the outcome of these pulmonary infections. Gentamicin, however, was not detected in lung secretions after intramuscular injections. Some clinical improvements were observed after endotracheal infusions, however at the frequency of three times a day. Gentamicin also has systemic toxicity, specifically renal toxicity. So a noval and controlled drug delivery system is essential for these drugs in order to reduce the side effects and increase the beneficial effects.

The result obtained in our study indicates the higher accumulation of Gentamicin and Rifampicin in lungs and hence a new approach of antibiotic therapy for tuberculosis and nosocomial pneumonia could be investigated in order to modify the pharmacokinetics of Rifampicin and Gentamicin sulphate using niosomes.