CHAPTER 9

SUMMARY AND CONCLUSION

9.1 SUMMARY

The spread of tuberculosis (TB) has reemerged as an urgent health problem. Each year, about 2 million people in the world die as a result of the infectious disease caused by *Mycobacterium tuberculosis*. Current methods of treatment are far from optimal and better ones are being sought to overcome the increasing spread of TB and the problem of incompletely treated TB that contributes to the emergence of drug resistant strains. Since many patients with TB may have significant social problems, compliance with drug therapy is frequently difficult. The development of targeted drug delivery to the lungs as a means of treating TB is desirable for several reasons. Although TB is a systemic disease that can potentially affect any organ system, the lung is the major portal of entry for *Mycobacterium tuberculosis* and thereby the site of the initial immune response as well as an important site of reactivation disease. Technology for lung specific drug delivery systems is now at a point where aerosols and aerosols combined with liposomes and possibly timed-release methodology may offer advantages for more effective treatment and prevention of TB. Conventional antituberculer medications frequently have serious side effects. Although single drugs can be effective for prophylactic treatment of skin test converters, active disease must be treated using combinations of three or four drugs over a period of at least six to nine months to ensure that disease will not reoccur after treatment is discontinued and to prevent the emergence of resistant strains. Targeted delivery of new formulations, directly to the lungs, could result in high pulmonary levels relative to systemic levels. When the lung is clinically involved organ, supplementing the dose of agent delivered to the diseased lung will increasing effectiveness and decreasing toxicity and probably decrease the duration of the treatment. Another advantage is that this mode of delivery might make it easier to provide prolonged treatment. Improved targeted delivery approaches combined with development of new antituberculer drugs or with timed release formulations may reduce the frequency of dose delivery. This would be a major benefit in treating patients in whom it is hard to maintain effective compliance with treatment regimens. For example, longer intervals between treatments would make it easier to deliver directly observed therapy, which is an effective means of getting patients to complete a full course of treatment. Targeting the drug to the alveolar macrophages (AMs) would be a rational addition to current

antitubercular therapy, potentially enhancing efficacy and reducing toxicity. Pharmaceutical aerosols, >5 μm once deposited may be removed by macrophage action before the dose is delivered, thereby reducing the bioavailability of the drug. Whereas for an antituberculer compounds it is the target region, as the pulmonary *Mycobacterium tuberculosis* infection is characterized by AMs containing large numbers of bacilli. Targeting the drug to AMs would be a rational addition to current therapy, potentially enhancing efficacy and reducing toxicity. Thus, a drug delivery system targeted to the AMs might be effective and has yet to be evaluated by direct administration to the lungs.

Liposomes are used as carriers for drugs and antigens. Liposomes can prolong the duration of drug exposure, acting as a slow-release reservoir. This has been demonstrated in a number of studies. Liposomes can protect a drug against degradation (for example metabolic degradation). Conversely, liposomes can protect the patient against side effects of the encapsulated drug. Liposomes can be used to deliver biological agents either entrapped within the internal aqueous compartments, reconstituted in the lipid bilayer, or attached to the outer surface. Liposomes are artificial lipid vesicles composed of concentric lipid bilayers that alternate with aqueous compartments. They have permeability properties similar to those of biological membranes. The liposomal encapsulation has been shown to reduce the entry of the agent into the systemic circulation, compared with free drug and provide distribution throughout the airspace of the lung. One of the major advantages of liposomes over other carrier delivery systems (Microspheres, Niosomes etc.) of drugs is that they can be prepared from materials for which there is considerable data available regarding their fate *in vivo*.

One of the perceived benefits of liposomes as a drug carrier is based on their ability to alter favorably the pharmacokinetic profile of the encapsulated species and thus provide selective and prolonged pharmacological effects at these sites of administration. The resulting decrease in the frequency of drug dosing will significantly improve the quality of life for patients and at the same time reduce healthcare cost. The selective and controlled release of the drug is also expected to reduce or eliminate hypersensitivity and systemic toxicities. The challenging aspect still remains unanswered are the mode of delivery for liposomally encapsulated drug. Metered dose inhalers (MDI) are currently being reformulated as a result of the ban being implemented throughout the world by the

United Nations on the use of chlorofluorocarbons (CFCs) to meet this challenge, one such alternative is the development of new and improved "Dry Powder Inhaler (DPI) system that will allow inhalation administration of all drugs presently delivered with MDIs. With constrain of propellant phase out and short-term stability of liposomal aqueous dispersion the most viable alternative would be to deliver the liposomally encapsulated drug in dry form.

Pharmaceutical aerosols, 1-5 μm once deposited may be removed by macrophage action before the dose is delivered, thereby reducing the bioavailability of the drug. Whereas for an antituberculer compounds it is the target region, as the pulmonary Mycobacterium tuberculosis infection is characterized by AMs containing large numbers of bacilli. Targeting the drug to AMs would be a rational addition to current therapy, potentially enhancing efficacy and reducing toxicity. Thus, a drug delivery system targeted to the AMs might be effective, but has yet to be evaluated by direct administration to the lungs. Isoniazid and Rifampicin are the first line antituberculer agents selected for liposome encapsulation as the current treatment of pulmonary tuberculosis involves prolonged oral administration of large systemic doses of combined antibiotics, which are associated with unwanted side effects and poor compliance. It was hypothesized that liposomal INH and RFP will control the release rate of the drug for longer duration at localized site and is expected to reduce systemic side effects and frequency of dosing. Hence, this investigation was focused on the pharmaceutical development of liposomal dry powder inhaler drug formulations of selected drugs evaluation, optimization of flow and dispersion (deaggregation) characteristics of the formulations under development and the evaluation of the selected formulations in animals.

The drug content and the excipients of liposomes were analyzed by the reported analytical method with suitable modification whenever necessary to meet the requirement of this investigation. The method was standardized for estimation of drugs (INH and RFP) under study, Hydrogenated Soyaphosphatidylcholine (HSPC) and cholesterol (CHOL) contents. The ability of phospholipids to form a red colored complex with ferrothiocyanate in organic solutions was used to estimate HSPC. The method was found to obey beer/s law between 10-150μg/3ml concentrations of HSPC in chloroform. Complexation of CHOL with ferric chloride in acetic acid was the basis of the

colorimetric method used for estimating CHOL. The method was found to obey beer's law between 5-50 µg/ml. Calibration curves of INH and RFP were prepared by UV-Visible Spectrophotometric method. The method was found to be sensitive between 4-20µg/ml at the λ_{max} 261 nm and 334 nm for INH and RFP respectively. Absorbance of the standard solutions was measured at the absorbance maxima and plotted graphically to get calibration curve. Regression analysis of the data proved the linearity of plots in the concentration range used. The interference of formulation components were checked and found non-interfering at absorbance maxima of the drugs.

Multilamellar liposomes were prepared by TFH and modified REV method using alternative organic solvents. Percent drug entrapment obtained by both methods were compared and found that TFH method was optimum for both drug. Prepared liposomes were extruded through 2 µm polycarbonate membrane to reduce their particle size suitable for inhalation delivery. The optimizations of various process variables like Vacuum, hydration time, speed of rotation, no. of extrusion cycles, annealing time and separation of unentrapped drug and formulation variables like composition of solvent system, volume of solvent system, volume of hydration medium, drug: lipid ratio, composition of lamellae, choice of organic solvent for REV method and ratio of aqueous phase to organic phase were optimized. Percent drug entrapment was calculated as percent of drug initially added. The optimum lipid composition (PC: CHOL: charge ratio) of 10:0:0 and 9.5:0:0 5 for INH and 10:0:0 and 9.9:0:0.1 for RFP liposomes neutral and negatively charged liposomes respectively was found to be optimum bilayer composition. By comparing percent drug entrapment values obtained by TFH and REV method, liposomes prepared with TFH method and trans-membrane drug loading of INH found to posses better process parameters and desirable PDE values i.e. 63.71 % - INH13 neutral and 64.19 % - INH17 negatively charged liposomes. Similarly for RFP liposomes, TFH method found to posses better process parameters and desirable PDE i.e. 83.71 % - RFP13 neutral and 88.80 % - INH17 negatively charged liposomes.

In TFH method, vacuum of 15 inches of Hg was found to be optimum as differential solubilities of amphiphilic components of bilayer and drug in organic solvents are often encountered and must be taken into consideration in order to avoid crystallization of a single component during solvent-stripping operations. By increasing or decreasing

vacuum applied resulted in precipitation of drug or drug leakage in liposomes respectively. Composition of solvent system comprising Chloroform: methanol (2: 1) was optimum for both INH and RFP liposomes. Similarly for REV method, vacuum cycle development is also important for obtaining high drug entrapment. Solvent system volume of 10ml for 100mg lipid concentration, hydration time of 1 hour, speed of rotation of 120 rpm, no. of extrusion cycle - 3 for INH and 5 for RFP liposomes was found to be optimum for reducing the particle size of liposomes below 2 μm. A freeze-thaw cycle of liposomes were done by freezing the liposomal dispersion at -50°C for 1 h and thawing it at 30°C/60°C for ½ h, 3 cycle was found to be optimal and resulted in proper orientation of PC molecules and intimated packing of lamellae. In REV method, ethyl acetate alone resulted in distorted spherical vesicles due to formation of unstable biphasic system upon contact with aqueous phase while ethanol alone resulted in high PDE due to formation of monophasic system upon contact with aqueous phase. However, drug leakage was observed due to presence of traces of ethanol leading to disruption of bilayer. In case of ethyl acetate: ethanol (1:1) combination, proper spherical vesicles and high PDE was observed. Also, aqueous phase to organic phase ration was found optimum to be 1:5 and 1:4 for INH and RFP respectively. It may be due to proper emulsification and formation of fine droplet surrounded by phospholipids i.e. liposomes, with uniform size, shape and high PDE. Ultra-centrifugation at higher G value ($5.33 \times 10^6 \times g$) was found to be efficient separation technique for unentrapped INH and RFP in liposomes.

Drug entrapment in liposomes was determined after treatment of liposomes with modified bligh-dyer two-phase extraction procedure. PC was quantified by ion-pair complexation with ferric ammonium thiocyanate while CHOL was quantified by complexation reaction with ferric chloride in acetic acid. All batches of liposomes were observed under Olympus (BX40F4, Japan) microscope with polarizing attachment to study their shape and lamellarity. Lamellarity was confirmed by presence of maltease crosses in liposomes. The vesicle size was determined by laser light scattering using Mastersizer (Malvern Instruments, UK). Liposomes prior to extrusion had a greater mean size which on extrusion through 2-μm polycarbonate membrane made suitable for lung delivery (<5μm). The trapped volume as μl per μ moles of PC was also determined by Karl Fischer method and was proportionally related to the liposomal size particularly for

hydrophilic drug (INH), which is present predominantly in the inner aqueous compartment.

Pre-loading of INH in to liposomes was found low; however the developed INH-EDTA and post-loading (Transmembrane loading) gave better PDE. The advantage of transmembrane drug loading was that the lipid concentration in the aqueous carrier used for incubation had no significant influence on the internalization capacity. Hence by concentrating the liposomes in the aqueous carrier favorably influence the entrapment yield and reduce the amount of residual non entrapped substances to be recovered and reused. However for the ease of extrusion of liposome a lipid concentration of 100mg/ml was selected in our experiments.

For preparing LDPI formulations, liposomes were prepared using cryoprotectant in such a way to preserve the liposomal integrity after lyophilization. The dispersion obtained was frozen at –40°C and lyophilized for 48 hours (Heto Drywinner model DW1 0-60E, Denmark). After lyophilization, the ability of various sugars like lactose, trehalose and sucrose with and without low density excipient (Hydrolyzed gelatin - HG) to preserve the permeability barrier in freeze dried vesicles was compared. The amount of drug retained retained (PDR) was determined. Sucrose with HG was found to be best cryoprotectants when sucrose present on both sides of the bilayers. Sucrose was selected for advantageous of being low cost and easy availability. The mass ratio of sucrose with HG required for cryoprotection depends upon the lamellarity and size of the vesicles and the saturation of polar head groups of the bilayer by the drug or its formulation components. A mass ratio of 1:1:0.5 (lipid: sucrose: Hydrolyzed gelatin) for INH liposomes and 1:0.5:0.5 (lipid: sucrose: Hydrolyzed gelatin) for RFP liposomes was found to be optimum to preserve their contents. The percent drug retained obtained for optimized liposomal dry powder after rehydration for INH and RFP showed insignificant difference between PDE to PDR of liposomal dispersion after freeze-drying.

To prepare LDPI formulations, a series of experiments were done. INH LDPI formulation were developed using lactose blend of 63-90 μm sieved Pharmatose 325M and 5% of Sorbolac 400 (<32μm) as fines in liposome: lactose mass ratio 1:1 were found to be optimum. RFP LDPI formulation were developed using lactose blend of 63-90 μm sieved Pharmatose 325M and 10% of Sorbolac 400 (<32μm) as fines in liposome: lactose mass

ratio 1:2 were found to be optimum. The addition of fines and order of mixing of fines were found to influence the FPF with significantly different device fractions for both drug formulations. The FPD (µg), FPF (%), Dispersibility (%) and Emission (%) at 60 L/min flow rate using Rotahaler (Cipla, India) as dispersing device were determined. Twin stage impinger (TSI) was used for particle characterization of fine particle fraction (FPF) as described in British Pharmacopoeia. FPF of INH LDPI formulations using Rotahaler (Cipla, India) as delivery device at 60 L/min was found to be 35.9 ± 0.8 % and 32.2 ± 0.8 % for INH70 and INH71 respectively. FPF of RFP LDPI formulations at 60 L/min were found to be 26.3 ± 0.6 % and 29.6 ± 0.7 for RFP70 and RFP71 respectively. The data derived from TSI reflect the fraction of inhalation dose likely to deposit in the lower airways. The potential LDPI formulation particle size distribution and fine particle fraction were measured using Next Generation Impactor (NGI). The FPF obtained by NGI were comparable to TSI measurements 32.11 % for INH70, 29.95 % for INH71, 24.71 % for RFP70 and 27.42 % for RFP71 LDPI formulations.

Moisture content of LDPI formulation is an important determinant for stability on storage and deaggregation upon inhalation. The liposomal dry powder had low moisture content (<2 %) confirms their low aggregation tendency. Liposomal dry powder made using sucrose, trehalose and lactose alone had shown higher tendency to absorb water into their amorphous structure. While LDP made using sugar and hydrolyzed gelatin mixture had showed lesser tendency to absorb water. Also, in development of LDPI formulations, evaluation of flow and dispersion characteristics of the formulations are of critical importance. The flowability and floodability expressed by angle of repose (32.1 to 39.7°), compressibility index (17.1 to 24.3) of LDPI formulations falls under category of good and floodable. The VMD for the potential liposomal dry powders were in the range of 15.4 -17.4 µm. Though, the VMD of liposomal dry powders were observed to be > 5 µm the administration of the low density particles (less than about 0.4 g/cm3) to the lung by aerosolization permits deep lung delivery of relatively large diameter therapeutic aerosols, for example, greater than 5 µm in mean diameter. This is evident from the calculated theoretical Mean Aerodynamic Diameter (MADt) are in relationship with the obtained MMAD of potential LDPI formulations (INH70 4.23, INH71 4.53, RFP70 4.12 and RFP45 4.71 µm).

The stability studies were carried out as per ICH guidelines for countries falling under zone III (hot, dry) and zone IV (very hot, humid). The product in its final packing (gelatin capsule in HDPE bottles with PVC coated aluminum foil cap) was stored separately for each sampling point and analyzed for the percent drug preserved entrapped. The percentage of drug remained entrapped (PDR) for LDPI formulations of INH after six months accelerated storage (40°C 75% RH) was 66.46 % (INH70) and 78.24 % (INH71) was below the acceptable level, hence as per the guideline recommendation one can not assign a shelf-life of 18 months. However, stability for one year at intermediate storage condition (30°C 65% RH) was 98.35 % (INH70) and 97.35 % (INH71), and at controlled room temperature storage (25°C) was 100.36 % (INH70) and 99.46 % (INH71) ascertains a shelf-life of 18 months at CRT. It is apparent that the LDPI formulations prepared using HSPC possessed glass transitions temperature < 35°C, the cause for the fusion of these liposomes at accelerated conditions might be due phase melting of lipid bilayer above this temperature. No major difference was observed due to effect of charge on stability for INH LDPI formulations. Similarly, the PDR of RFP70 was observed to be 93.45 % and 90.04 % for RFP71, and at controlled room temperature storage was 94.36 % (RFP70) and 91.61 % (RFP71).

The growth in vesicle size of liposomes upon rehydration was determined from changes in liposomal size for all the batches prior to and after storage at controlled room temperature and intermediate storage and was found to be insignificant when compared with student's t-test. The increase in particle size and the decrease in PDR with the increase in the temperature of storage were observed due to fusion of liposome for both drugs. This fusion of liposome can be controlled by selection of higher glass transition temperature lipid composition. The FPF of INH LDPI formulations results were observed to be in parallel to the results of liposomal size on rehydration i.e. at accelerated storage the FPF found to decrease on prolonged storage (Anova: Single Factor $P>0.05$). However there is no significant difference in FPF on intermediate and CRT storage was observed for a year (t-Test: Paired $P<0.05$). It can be concluded that change in liposomal mean size is non significant before and after storage up to 12 months intermediate storage and controlled room temperature storage for both INH and RFP LDPI formulations. The slight increase in the liposome size may be due to aggregation on storage that is of

insignificant level. The formulations were also observed for caking and discoloration of LDPI formulations. Marginal caking or discoloration was observed for batches stored under accelerated storage conditions after 3 months. These types of observations were less visible at intermediate storage and controlled room temperature storage of both LDPI formulations.

Comparative diffusion studies were was carried out of plain drugs (INHPD & RFPPD and LDPI formulations using self designed and validated diffusion cell for a period of 12 to 24 hours. The non-linearity of the graph suggests that the diffusion pattern does not follow zero order kinetics of release. The regression coefficients (0.9422 – 0.9873) of the data of percent drug diffused Vs Root t suggest that a linear relationship exists between percent drug diffused and Root t confirming the release obeys Higuchi's diffusion controlled model.

The mean flux values of PD is found to be two times higher than those of LDPI formulations underscore sustained drug diffusion from liposomal encapsulated drug formulations. Similarly, the diffusion coefficient of the PD is four times higher than that of LDPI formulations, confirming a sustained diffusion following liposomal encapsulation of these drugs.

On comparing the flux of both neutral and negatively charged liposomal DPI formulations, negatively charged liposomal DPI formulations found to diffuse little less than neutral formulations (INH70 and RFP70). It may be due to ionic interaction of these drugs with charge present in liposomal membranes and thus retarding the drug diffusion. The diffusion coefficient is governed by the concentration of free drug in the donor compartment and depends on the rate of drug diffusion from liposomes. In the in vitro alveolar macrophage uptake studies liposomes demonstrated an apparent maximal uptake of 73 ± 8.7% at 20 µg/ml. No significant differences were observed in the uptake of different types of liposomes size and charge.

The drug release studies in rats were performed by intratracheal instillation. Solutions containing non-encapsulated drug or liposome-encapsulated drug were prepared by rehydration (30 min) of powder with distilled water and were instilled into the cannulated trachea. Broncho alveolar lavage (BAL) was performed on anaesthetized and recannulated animals with 12 ml PBS pre warmed to 37°C. This broncho alveolar lavage

yielded between 7 to 11 ml liquid. The lungs and the portions of trachea below the instillation site were excised and homogenized (LH) in 10 ml of PBS containing 1% Triton – X – 100.

When the concentration-time profiles were examined upto 12-24 h post-instillation, higher T_{max} for liposome formulations (INH70, INH71, RFP70 and RFP71) was observed than of plain drug. There was an increase in AUC $^{24h}_{0}$ for liposomal formulations compared to plain drug. The higher T_{max} and AUC $^{24h}_{0}$ observed for liposomal formulations confirm prolonged drug retention in lung than plain drug assuring a better therapeutic activity. The in vivo concentration-time profile showed the kinetics of LDPI formulations was altered by liposomal encapsulation depending on composition of bilayers. Site targeting index (STI) was observed to be higher for LDPI formulations (INH70- 1.08, INH71-1.96, RFP70-1.73 and RFP71-1.54) than for plain drugs (INHPD-0.19 and RFPPD-0.94. STI gives an accurate measure on how effectively the drug is actually delivered to intended site of action. Hence, the free drug was rapidly absorbed from the lung to systemic circulation; while the liposomal encapsulated drug remained in the lung for a prolonged period of time. Similarly, the higher Site-exposure enhancement factor (SEF) values of LDPI formulations compared to plain drug corroborated the effectiveness of the liposomal drug delivery. A good delivery system not only increases exposure of the active agent at the target site, but also decreases the corresponding systemic exposure. Targeting enhancement factor TEF is the most rigorous measure and used in quantifying the targeting produced by a delivery system. It compares not only concentrations, but also concentrations along a time period and it compares actual, active drug concentration, both at target and systemic sites. Higher TEF values were obtained for LDPI formulations than plain drug confirms that the liposomal drug delivery to lung not only increases exposure of the active agent at the target site, but also decreases the systemic exposure.

When toxicities induced by free and liposomal drugs were performed by monitoring the levels of SFOT, SGPT, and ALP before and on 24 hour serum samples. The encapsulation of INH and RFP in liposomes reduced the levels of SFOT, SGPT, and ALP compared to those observed for free drugs. The differences in the levels induced by free and liposomal drugs (INH and RFP) were highly significant (*P*, 0.05) The levels hepatic

enzymes (SGPT and ALP) demonstrated that encapsulation of INH and RFP in liposomes reduced their toxicities significantly (P, 0.05) compared to those of free drugs.

Different portions of broncho-pulmonary tree possess different characteristics; it is possible that drug diffusion from liposomal DPI formulation is affected by its distribution within the lung and later altered by mucociliary transport and other mechanisms. Animal studies reported till date has utilized instillation of liquid formulations in order to obtain accurate dosimetry. Such results depend upon the spreading of the instilled dose within the lung. The distribution and absorption of inhaled aerosols in the lungs and airways are different from those of instilled liquid and it is possible that diffusion kinetics of aerosol formulations in humans may differ considerably from diffusion kinetics of the instilled formulations in animals. Additionally, the size and aerodynamic properties of human airways may result in a significantly different distribution and rehydration of aerosolized liposomes to rodent animals, which may affect observed diffusion kinetics, duration, onset and intensity of effect.

Findings of these studies conclusively demonstrated superiority of LDPI formulations over plain drug by exhibiting maintenance of effective drug concentrations in the lung tissues for prolonged time, slow clearance from the lung and reduced toxicities.

9.2 CONCLUSIONS

Liposomal drug delivery to lung offers a number of advantages over conventional drug delivery systems such as localization of drug within the lungs, prolonged and controlled drug release, and enhanced cellular drug uptake. For achieving these objectives, liposomally entrapped drug must be obtained in a stable form that can be delivered conveniently and selectively to the targeted site in the lungs.

TFH and REV method were used for the preparation of neutral and negative charged liposomes of INH and RFP. HSPC containing liposomes were found to be more stable than SPC containing liposomes. It may be due to rigidity offered by HSPC in liposomal membranes. No significant difference in PDE was observed between neutral and negatively charged liposomes. Prepared liposomes were characterized for size, shape, lamellarity and trapped volume and then subjected to lyophilization using sucrose with hydrolyzed gelatin as a cryoprotectant. Maximum PDR was obtained at lipid: sucrose:

HG mass ratios of 1:1:0.5 for INH liposomes and 1:0.5:0.5 for RFP liposomes. Prevention of drug leakage may be due to interaction of sucrose and hydrolyzed gelatin with the polar head groups of the bilayers. Lyophilized liposomes were formulated as LDPI formulations using sieved Pharmatose 325M (63-90μm) and Sorbolac 400 (32 μm) as carriers for INH and RFP LDPI formulations. The effect of addition of fines and order of mixing fines were found to have significant influence ($p<0.05$) on in vitro lung deposition of drug from LDPI formulations. At 5-10% level of fines, high-energy adhesion sites (HA) of lactose bind strongly to the fines and low-energy adhesion sites (LA) allow the formation of more reversible bonds with liposomal drugs. This results in efficient deaggregation of liposomal drug from the carrier. Prepared LDPI formulations were characterized for flow properties, such as angle of repose, tapped density, compressibility index, and dispersibility index. In vitro lung deposition studies were carried out for these LDPI formulations using Twin stage impinger. Fine particle fraction (FPF), Percent emission, Fine particle dose (FPD), dispersibility and Effective index were calculated for the formulations. The percent emission of 78.9 – 84.1 % suggests more effective emission of the liposomal DPI deposition in the lung (FPF values-26.3% - 35.9%). It was concluded from these observations that carrier particle size plays important role in liposomal DPI formulation.

The stability studies carried out as per ICH guidelines for countries falling under zone III (hot, dry) and zone IV (very hot, humid). It was apparent that the LDPI formulations prepared using HSPC possessed glass transitions temperature < 35°C, the cause for the fusion of these liposomes at accelerated conditions might be due phase melting of lipid bilayer above this temperature. No major difference was observed due to effect of charge on stability for INH LDPI formulations. The growth in vesicle size of liposomes upon rehydration was determined from changes in liposomal size for all the batches prior to and after storage at controlled room temperature and intermediate storage and was found to be insignificant. The increase in particle size and the decrease in PDR with the increase in the temperature of storage were observed due to fusion of liposome. The FPF of INH LDPI formulations results were observed to be in parallel to the results of liposomal size on rehydration i.e. at accelerated storage the FPF found to decrease on prolonged storage. However there is no significant difference in FPF on intermediate and CRT storage was

observed for a year. It can be concluded that change in liposomal mean size is non significant before and after storage up to 12 months intermediate storage and controlled room temperature storage for both INH and RFP LDPI formulations. The slight increase in the liposome size may be due to aggregation on storage that is of insignificant level. Marginal caking or discoloration observed for batches stored under accelerated storage conditions after 3 months and were less visible at intermediate storage and controlled room temperature storage of both LDPI formulations. Comparative diffusion studies suggest that a linear relationship exists between percent drug diffused and Root t confirming the release obeys Higuchi's diffusion controlled model. The mean flux values of PD were found to be two times higher than those of LDPI formulations. The in vitro alveolar macrophage uptake studies liposomes demonstrated an apparent maximal uptake of 73 ± 8.7% at 20 μg/ml. No significant differences were observed in the uptake of different types of liposomes size and charge.

The drug release studies in rats were performed by intratracheal instillation showed concentration-time profiles up to 12-24 hours post-instillation there was a rank order decrease in Cmax from plain drug to the formulation containing neutral and negative charge in their composition. There was an increase in AUC $^{24h}_{0}$ for liposomal formulations compared to plain drug, the percent increase in the AUC $^{24h}_{0}$ for negatively charged liposomal formulations were more compared to neutral liposomal formulation suggesting that negatively charged liposomal formulations retain more in the lung. The STI reveals how effectively the drug is actually delivered to its intended site of action. Thus the free drug was rapidly absorbed from the lung to systemic circulation, while the liposomal encapsulated drug remained in the lung for a prolonged period of time. Similarly the higher SEF values of LDPI formulations in comparison to drug alone show the effectiveness of the liposomal drug delivery. A good delivery system not only increase exposure to the active agent at the target site, but also decreases the corresponding systemic exposure. The lower levels of serum SGPT, SGOT and alkaline phosphatase were observed as compared to the plain drugs administered through lung demonstrated that encapsulation of INH and RFP in liposomes reduced their toxicities significantly compared to those of free drugs.

Maintenance of effective drug concentrations in the lung tissues for prolonged time, less clearance from the lung and lower levels hepatic enzymes demonstrates the superiority of LDPI formulations over plain drug solutions.

The studies support that carrier based (liposomal dry powder inhaler) pulmonary drug delivery for treatment of tuberculosis exhibits prolonged drug retention at targeted site and reduces the systemic exposure. Hence, it is expected to maximize the therapeutic index, reduce the systemic side effects, frequency of dosing and dose, and probably cost of therapy. However, the role of liposomal drug dry powder inhaler formulations in treatment of tuberculosis can only be settled after experiments on at least two animal species followed by extensive clinical trials. This investigation has only provided preliminary evidence that delivery of carrier based dry powder inhaler of antitubercular agents can confine drug primarily to lung (alveolar macrophage) using a liposomal dry powder Inhaler delivery system and hence expected to treat the disease better and can also avoid the development of drug resistance.

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