CHAPTER 9

SUMMARY AND CONCLUSION

9.1 Summary

The respiratory tract is one of the oldest routes for the administration of drugs. Anesthetics, aerosolized drugs, smoke or steam have been inhaled for medical purposed for centuries. Over the past decades inhalation therapy has established itself as a valuable tool in the local therapy of pulmonary disease, such as asthma or COPD. Historically, the evolution of inhalation therapy can be traced in India 4000 years ago, where the leaves of the Atropa Belladonna plant, containing atropine as bronchodilator, were smoked as a cough suppressant. The development of modern inhalation therapy began in the nineteenth century with the invention of the glass bulb nebulizer. The development of the first pressurized metered dose inhaler (pMDI) for asthma therapy in 1956 was a major advance in the use of aerosols for drug delivery to the lungs. However, the required hand-lung coordination of the patient and the use of environmentally damaging CFC propellants, are major drawbacks of the traditional pMDIs. Dry powder inhalers (DPI) were introduced to overcome these drawbacks. From the first introduction of DPI in 1971 (Spinhaler), several DPI's have been added to the collection of available inhalers. DPI's represent a significant advance in pulmonary drug delivery technology for humans. They are breath-controlled and so coordination problems have been over come. DPI's are also potentially suitable for the delivery of a wider range of drugs, such as anti-asthmatics, peptides and proteins. DPI's can also deliver a wide range of doses from 6 μg to more than 20mg via one short inhalation. Direct application of drugs to the lungs reduces the exposure of drug to systemic circulation and potentially minizes adverse effects. Lower dosage regimens may provide considerable cost saving especially with expensive therapeutic agents. In spite of these advantages and the widespread use of therapeutic

aerosols, there are several deficiencies associated with drug delivery to the respiratory tract. A significant disadvantage of may exhisting inhaled drugs is the relatively short duration of resultant clinical effects and most medications in aerosol form require inhlation at least 3-4 times daily. This often leads to poor patient compliance with the therapeutic regime and increases the possibility of associated side effects. A reduction in the frequency of dosing would be convenient, particularly for chronic diseases. Sustain release of such drugs in the lung would be particularly beneficial since they could be delivered to and retained at the targeted receptors for a prolonged period and thus minimize the biodistribution throughout the systemic circulation.

The use of liposomes in pulmonary delivery was first investigated as a potential treatment for respiratory distress syndrome. 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 slective and prolonged pharomacological effects at the site of adminstaion. The resulting decrease in the frequency of drug dosing will significantly improves the quality of life of patients and at the same time may reduce the healthcare cost. The selective and controlled release of the drug is also expected to reduce or eliminate hypersensitivity and systemic toxicities. The challege will remain to deliver these liposmal encapsulated drug to the lungs as CFC being banned and short term stability of liposomal aqueious dispersions. The alternaive would be to deliver these drugs in dry form by development of LDPI formulations.

AMK is a broad-spectrum and potent aminoglycoside with limited clinical use due to high dose requirement, renal and audio-vestibular apparatus toxicity. Major drawbacks associated with the use of earlier or conventional liposomal **formulations** are the

tendency of liposomes to leak drug while in circulation, the extensive uptake of these liposomes by tissues of reticuloendothelial systems (RES) and inability of liposomes to extravasate into infected tissue. Therefore localized liposomal AMK delivery was thought for the treatment of cystic fibrosis infections in lungs. Liposomal encapsulation of AMK will give required release of drug for longer time duration at localized site, thereby will reduce the chances of systemic side effects and reduce frequency of dosing.

There has been a dramatic rise in the number of invasive fungal infections in immunocompromised patients in recent years. Accordingly, there is an urgent need to improve the treatments for invasive fungal infections because; the overall prognosis for patients with these infections remains poor. AMB is a broad-spectrum and potent antifungal agent, but its clinical use is sometimes limited due to adverse reactions, such as renal toxicity, hypokalemia and anemia. A promising approach to the treatment of invasive fungal infections is the use of liposomal AMB. LDPI formulation wass chosen as means to stabilize the liposomal system and localized liposomal AMB delivery was thought as an alternative treatment of invasive lung fungal infections. It was hypothesized that liposomal AMB 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 stable liposomal AMB DPI formulation for high pulmonary deposition.

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 (AMK and AMB) under study, Hydrogenated Soyaphosphatidylcholine (HSPC) and cholesterol

(CHOL) contents. Calibration curves of AMK and AMB were prepared by UV-Visible Spectrophotometric method. The method was found to be sensitive between 10-60μg/ml and 2-8μg/ml and the λ_{max} used were 350 nm and 410 nm respectively. 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 and sulphuric acid was the basis of the colorimetric method used for estimating CHOL. The method was found to obey beer's law between 5-100 μg/ml in glacial acetic acid. 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 modified REV method using alternative organic solvent i.e. Ethyl acetate: ethanol (1:1) and aqueous phase to organic phase ratio of 1:5 were optimum. Prepared liposomes were extruded 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 1:2:0.1 for AMK liposomes (negatively charged and positively charged liposomes) and 7:3:0.5 for negatively charged AMB liposomes and 5:5:0.5 for positively charged AMB liposomes was found to optimum bilayer composition. By comparing percent drug entrapment values obtained by TFH and REV method, liposomes prepared with REV method resulted in high drug entrapment values i.e. 96.71 % - AMK31 negatively charged liposomes and 98.54 % - AMK32 positively charged liposomes. Similarly for AMB liposomes, REV method resulted in high PDE i.e. 95.84 % - AMB37 negatively charged liposomes and 87.92 % - AMB38 positively 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. Similarly for REV method, vacuum cycle development is also important for obtaining high drug entrapment. Composition of solvent system comprising Chloroform: methanol (2: 1) was optimum for AMK liposomes where as Chloroform: methanol (1:1) was found to be optimum for AMB liposomes. For AMB, chloroform: methanol (1:1) is found more suitable to prevent precipitation of AMB as AMB was added along with lipid phase & solubility is limited in both solvents and thus combination at 1:1 ratio is more suitable to prevent precipitation of drug crystals. Solvent system volume of 20ml, hydration time of 1 hour, speed of rotation of 120 rpm, no. of extrusion cycle - 5 for AMK and 7 for AMB liposomes was found to be optimum for reducing the particle size of liposomes below 2

μm. Annealing time of 1 hour for hydrophilic drug AMK) and 5 hours for hydrophobic drug (AMB) were found to be optimum 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. 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.

Dialysis was found to be efficient separation technique for unentrapped AMK and controlled centrifugation at low speed (3000 rpm) was efficient for separation unentrapped AMB 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 presence of 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 for

being 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 (AMK), which is present predominantly in the inner aqueous compartment.

For preparing LDPI formulations, liposomes were prepared using cryoprotectant containing buffer solution 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, maltose, dextrose, trehalose and sucrose to preserve the permeability barrier in freeze dried vesicles was compared. The amount of drug retained entrapped (PDR) was determined. Trehalose and sucrose were found to be best cryoprotectants when present on both sides of the bilayers. Sucrose was selected for advantageous of being low cost and easy availability. The mass ratio of sucrose required for cryopreservation 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 1:4 for AMK liposomes and 1:5 for AMB liposomes was found to be optimum to preserve their contents.

To prepare LDPI formulations, a series of experiments were done. AMK LDPI formulation were developed using lactose blend of 63-90 μm sieved Pharmatose 325M and 10% of 500 # sieved (<25μm) Sorbolac 400 as fines in liposome: lactose mass ratio to be 1:5. AMB LDPI formulations were developed using lactose blend of Sorbolac –400 and 10% of 500 # sieved (<25μm) Pharmatose 325M as fines in liposome: lactose mass ratio of 1:6. From the two devices described in British Pharmacopoeia for particle

characterization of fine particle fraction (FPF), liquid stage impinger (TSI) was used. The data derived from TSI reflect the fraction of inhalation dose likely to deposit in the lower airways. 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 30, 60 & 90 L/min flow rate using Rotahaler (Cipla, India) as dispersing device were determined. FPF of AMK LDPI formulations using Rotahaler (Cipla, India) as delivery device at 30, 60 and 90 L/min were found to be 21.8 ± 2.2 % and 24.6 ± 2.4 %; 25.9 ± 1.8 % and 29.2 ± 2.1 % and 29.5 ± 2.6 and 34.2 ± 2.0 for AMK69 and AMK70 respectively. FPF of AMB LDPI formulations 30, 60 and 90 L/min were found to be 23.1 ± 1.5 percent and 17.3 ± 2.2 percent; 25.3 ± 1.8 percent and 19.6 ± 1.5 percent and 28.4 ± 2.1 percent and 22.9 ± 1.9 percent for AMB77 and AMB78 respectively. Thus liposomal charge; carrier size, addition of fines and order of mixing fines has significant effect ($p<0.05$) on in vitro deposition of drug from LDPI formulation. Moisture content determination is important for stability on storage and deaggregation upon inhalation. The developed formulations had low moisture content (<2 %) confirms their low aggregation tendency. 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 (27.1 to 29.7°), compressibility index (21.9 - 23.8) falls under category of good and floodable.

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 was stored separately for each sampling point and analyzed for the percent drug preserved

entrapped. T_{90} (it refers to the time period when the drug preserved entrapped is 90 %) for accelerated storage condition it was between 1.5 months to 2.5 months, for controlled room temperature storage condition it was between 3.5 months to 6 months and at refrigerated & freezer storage condition it was 9 months for AMK LDPI formulation and 12 months for AMB LDPI formulations. This may be due to presence of CHOL in the formulation increases the rigidity of membrane thereby reducing the drug leakage below phase transition temperature of the bilayers for both drugs. There was decrease in percent drug retained entrapped with the increase in the temperature of storage. No major difference was observed due to effect of charge on stability for AMK LDPI formulations. The slight increase in percent drug retained in AMB78 formulation (positively charged LDPI formulation) may be attributed due presence of more proportion of CHOL in the bilayer composition. The data of same batches when stored at controlled room temperature and accelerated storage reveals that marked decrease in percent drug retained for both formulation with exception of AMB77 LDPI formulation (negatively charged LDPI formulation). This may be due to at accelerated storage conditions, the fluidity of bilayer increase leading to leakage of drug from the bilayers. For AMB 77 LDPI formulation (negatively charged LDPI formulation), the more proportion of amphillic PC is present leading to re-encapsulation of leaked drug in to the bilayers while there was less proportion of PC in AMB77 LDPI formulation and more proportion of CHOL leading to more fluid bilayer and hence more drug leakage from AMB77 LDPI formulation.

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 refrigerated conditions after 3 and 6 months respectively and was found to be insignificant when compared with student's t-test. These formulations were observed for caking and discoloration. Caking and discoloration was noted under accelerated conditions after 3 months storage. This phenomenon was less intense at controlled room temperature storage and was not evident at refrigerated and freezer storage. Even the flow and dispersion properties of the formulation stored at controlled room temperature, refrigerated and freezer points were similar to initial.

Comparative in vitro diffusion studies were carried out for plain drug and potential LDPI formulations. These studies were carried out in self designed and validated diffusion cell consisting of donor and receptor compartment separated by an artificial membrane (dialysis membrane). These studies were carried out over a period of 12 to 24 hours. The receptor compartment was filled with phosphate buffer saline containing 1 mM EDTA and 10% sucrose solution (such that of original sugar concentration of AMK LDPI formulation) for AMK or mixture of PBS, 1mM EDTA and 10% sucrose solution (such that of original sugar concentration of AMB LDPI formulation) and Methanol (6: 4) for AMB in order to maintain the sink conditions. Similarly LDPI formulation of AMK (equivalent to 15 doses; 1000 μg x 15) and LDPI formulation of AMB (equivalent to 10 doses; 250 μg x 10) were dispersed in 1 ml (AMK) and 2ml (AMB) of distilled water. ANOVA was performed over all the mean percent drug diffused values at $p<0.05$ indicating that all the mean values are significantly different from each other.

Regression coefficients of the data of percent drug released Vs Root t lies between 0.9492 – 0.9928 indicating that a linear relationship exists between percent drug released and Root t suggesting the release obeys Higuchi's diffusion controlled model.

The mean flux values of PD is found to be four to five times higher than those of LDPI formulations indicating that LDPI formulations are potentially sustaining the drug release. Similarly, the diffusion coefficient of the PD is 7 to 8 times larger than that of LDPI formulations, confirming a sustained release diffusion following liposomal encapsulation of these drugs.

On comparing the individual formulations it was found that the flux of both positively and negatively charged liposomal DPI formulations, negatively charged liposomal DPI formulations sustains more drug than positively charged formulations (AMK69 and AMB 77).

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. It was then extracted and assayed by spectrofluorometric method for amikacin and spectrophotometer for Amphotericin B.

Amount of drug present in the LH was considered as the drug absorbed and available for the pharmacological response and the amount of drug present in the BAL was considered as drug not absorbed into the lung tissue but still retained in the bronchial spaces (in liposomally encapsulated form). Later represents a reservoir of drug that eventually would be absorbed by the lung tissue. After instillation of liposomal drug, 40-60% of the

drug was recovered in BAL during the first 6 hours, which decrease by 12 to 24 hours. Lung tissue recovery of drug, after administration of liposomes, increased with time (Figure 8.1 and Figure 8.2). The total drug balance between the percent drug released and percent drug still present in liposomes (estimated in BAL) was not 100 %. It was assumed that the amount of drug that could not be accounted might have either metabolized or systemically absorbed or both.

When the concentration-time profiles were examined up to 12-24 hours post-instillation there was a rank order decrease in C_{max} from plain drug to the formulation containing negative and positive charge in their composition. Accordingly T_{max} of all four formulations were found to be more than their respective plain drugs. Similarly, 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 positively charged liposomal formulation suggesting that negatively charged liposomal formulations sustains more in vivo release of drugs (i.e. drug absorbed). The presence of more proportion of CHOL and positive charge in AMB78 has resulted in least AUC compared to negatively charged formulation (AMB77). Similar type of observation was also observed for AMK LDPI formulations. Cholesterol is know to protect liposomes from in vivo destabilization, but up to an optimum concentration after which it contributes to the destabilization of the liposomes in vivo as revealed from AUC $^{24h}_{0}$ for AMB78 with 1:1 HSPC: CHOL ratio. The destabilization caused by the inclusion of cholesterol is more for hydrophobic drug like AMB. Thus the kinetics of LDPI formulations in lung is found to be dependent on drug's physicochemical property and on the composition of liposomes. The inclusion of CHOL is must for the physical

stability of liposomes however the biological stability its level to be optimum and a further increase leads to a relatively rapid release of the medicament.

When the $T_{1/2}$ values were examined, there was increase in $T_{1/2}$ values from plain drug to negatively charged liposomal DPI formulations while for positively charged liposomal DPI formulations it was similar or slight more than plain drug. 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 particularly negatively charged LDPI formulations (AMK69 and AMB78). It also confirms that inclusion of cholesterol in the liposomal membrane decreases the membrane permeability.

9.2 CONCLUSIONS

The ability of liposomes to encapsulate drug offers a number of advantages over conventional pulmonary 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.

TLE and REV method were used for the preparation of negative and positive charged liposomes of AMK and AMB. 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 positive and negative charged liposomes. Prepared liposomes were characterized for size, shape, lamellarity and trapped volume and then subjected to freeze drying studies using sucrose as a cryoprotectant. Maximum PDR was obtained at lipid: sucrose mass ratios of 1:4 for AMK liposomes and 1:5 for AMB liposomes. Prevention of drug leakage may be due to

interaction of sucrose 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 AMK and AMB LDPI formulations respectively. The effect of addition of fines (10% w/w) 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 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 AMK69 (negatively charged LDPI) was found to be better (88.9% v/s 84.4%) than the AMK70 (positively charged LDPI) and lower FPF compared to positively charged LDPI formulation (AMK70) (25.9% v/s 29.2%). It suggests efficient dispersion of AMK69 from the device but deposition of more proportion of the dispersed powder in the upper respiratory tract. On the contrary, the percent emission of AMB77 (negatively charged LDPI) was found to be better (89.1% v/s 83.1%) than the AMB78 (positively charged LDPI) suggestive of more effective emission of the liposomal drug but interestingly deposition of more proportion of the dispersed powder in the lung (FPF values-25.3% v/s 19.6%) than AMK LDPI formulations. It may be due to turbo-electrification or charge generation in liposomal powder during dispersion via the

Rotahaler. Difference among both drugs LDPI formulations may be due to carrier particle size difference. AMK LDPI contains 63-90 µm sieved Pharmatose 325M as carrier; while AMB LDPI formulation contains Sorbolac 400 (<32 µm NLT 90%) as carrier. It can be concluded from these observations that carrier particle size plays important role in liposomal drug lung deposition from LDPI in presence of charge on liposomal surface. Higher device fractions of positively charged LDPI formulations (AMK70 and AMB78) are suggestive of some interaction within device it self.

LDPI formulations were evaluated for drug retention studies using sampling points according to ICH guidelines at freezer (-20°C), refrigerated (2-8°C), controlled room temperature (25°C ± 2°C, 60 ± 5 % RH) and accelerated condition (40°C ± 2°C, 75 ± 5% RH). After 9 months of storage of AMK LDPI formulations & 12 months storage for AMB LDPI formulations did not show significant ($p<0.05$) PDR at freezer and refrigerated conditions. It may be due to presence of CHOL in the formulation increasing the rigidity of membrane thereby increasing PDR below phase transition temperature of the bilayers for both drugs. PDR data of same formulations, when stored at controlled room temperature and accelerated storage, show significant decrease in PDR for AMB78 formulation compared to AMB77 LDPI formulation (66.67 % v/s 60.72%). At accelerated storage conditions, the fluidity of bilayer increases leading to leakage of drug from the bilayers. For AMB 77 LDPI formulation (negatively charged LDPI formulation), presence of more proportion of amphiphilic PC (70% v/s 50%) leads to re-encapsulation of leaked drug in to the bilayers; while there was less proportion of PC in AMB78 LDPI formulation (50% v/s 70%) and more proportion of CHOL (50% v/s 30%) leading to more fluid bilayer and hence more drug leakage from AMB78 LDPI formulation. The

increase in the shelf life with increase in the lipophilicity of drug is indicative of better potential for the delivery of hydrophobic drug entities by this delivery system.

LDPI formulations were subjected to in vitro drug diffusion studies using dialysis membranes (cut off point 12000 Daltons). In vivo drug diffusion studies were also carried out in rats by intra-tracheal instillation of liposomal suspensions compared to plain drug solutions. Maintenance of effective drug concentrations in the lung tissues (LH) for prolonged time and less rapid clearance from the lung perfusate (BAL) demonstrates the superiority of negatively charged LDPI formulations over plain drug solutions. The inclusion of negative charge in the liposomal formulations increases the pulmonary half-life of liposomally encapsulated drugs i.e. for AMK LDPI formulations, 4.3 hrs to 14.8 hrs and for AMB LDPI formulations, 5.2 hrs to 15 hrs. The results of investigations conclusively demonstrate that negatively charged LDPI formulations beneficially change pharmacokinetics of liposomally encapsulated AMK and AMB. Hence, LDPI formulations of AMK and AMB developed in this investigation are expected to maximize the therapeutic index, reduce systemic toxicities, reduced frequency of dosing and probably dose and cost of therapy. However, the role of developed LDPI formulations in clinical practice can only be settled after experiments on another two animal species followed by clinical trial.