Lung Delivery for Pulmonary Tuberculosis

Chandrakant Hingankar
T.Y.B.Pharm.
Pharmaceutical Sciences & Technology Department

Samir Joshi
T.Y.B.Pharm.
Pharmaceutical Sciences & Technology Department

Abstract

Tuberculosis (TB) is the second most deadly infectious disease caused by Mycobacterium tuberculosis. Though TB can be systemic, it most commonly affects the lungs (pulmonary TB). Despite potentially curative pharmacotherapies being available for over 50 years, the length of the treatment and the pill burden can hamper patient lifestyle. Thus, low compliance and adherence to administration schedules remain the main reasons for therapeutic failure and contribute to the development of multi-drug-resistant (MDR) strains. To overcome these problems novel drug delivery approach is being investigated. Increasing number of studies are being performed for the pulmonary administration of drugs using a variety of different inhalation techniques. The obvious advantages of inhaled therapy include direct drug delivery to the diseased lungs in pulmonary TB, targeting to alveolar macrophages (AMΦs) harbouring the mycobacteria, reduced risk of systemic toxicity and improved patient compliance. Controlled drug delivery through pulmonary route can be achieved by aerosolizable liposomes, microspheres, polymeric nanoparticles and Solid Lipid Nanoparticles (SLNs).

Keywords: Tuberculosis, Microspheres, Nanoparticles, Inhalation

1. Tuberculosis

1.1 Introduction

TB is a common infectious disease caused by Mycobacterium tuberculosis. TB infects almost all organs of the human body, but the most commonly the lungs (pulmonary TB). It is estimated that more than 80% of all disease originates as pulmonary TB.[1] The lungs are the main port of entry for Mycobacterium tuberculosis (Mtb) and an important site of disease manifestation which spreads to other organs. Mtb, the causative agent for TB, is a successful intracellular pathogen that targets and inhabits the professional antigen presenting cells (APC's), the alveolar macrophage (AMΦ) and the dendritic cells during the early stages of infection. The microorganism enters the human lung through droplet inhalation of as few as two to three bacilli, sufficiently small (1–5 μm) to be deposited in the alveolar space. AMΦs and dendritic cells play an important role in the initiation and maintenance of immune response against these pathogens. Bacteria are non-specifically phagocytised and are presented to T-lymphocytes. Mtb has evolved to evade most of the host-defense mechanisms enabling not only their intracellular survival but also replication within phagosomes of AMΦs. Mtb-infected phagosomes do not fuse with the lysosomes, thus escaping degradation by hydrolytic enzymes. Once inside the APCs, Mtb multiplies and spreads to other parts of the body depending on the immune response generated by the host.[2] TB is a systemic disease for which the most prominent extra-pulmonary sites of infection include tissue -Reticular Endothelial System (tissue-RES) containing organs like liver, kidneys, spleen, uterus and bones. Mycobacteria reach the alveoli, multiply intracellularly in AMΦs, and stealthily spread through the lymphatics to hilar or other lymph nodes, and then to the blood stream to infect other organs. Mtb continues to grow in the lungs for 2 to 12 weeks until in an immuno-competent host, cell-mediated immunity (CMI) develops. Tuberculosis that occurs after the initial exposure to the bacteria is referred to as primary TB. If the body is able to form scar tissue (fibrosis) around TB bacteria, the infection is contained in inactive state. Such an individual has no symptoms and cannot spread TB to other people. This condition is referred to as latent TB or secondary TB. About 90% of the people who get infected with TB develop latent TB. Sometimes, however body's immune system becomes weakened and TB bacteria break through the scar tissue and can cause active disease.[3] TB is a problem not only in the developing world and an increasing number of people in the developed world are contracting TB. During the last 20 years, increasing evidence has been presented that TB, especially multi-drug resistant (MDR) TB is on the rise in many countries. The total number of MDR-TB cases estimated to have occurred worldwide in 2008 is 4,40,000. If immediate measures are not taken to stop the spread of TB, WHO estimates that within the coming 20 years 92 million people will die from Mtb infections. One important reason for the current rapid increase in TB worldwide is the HIV epidemic, 9% of the 8 million new TB cases worldwide in 2000 and 12% of the deaths being attributed to co-infection with HIV.[1]

1.2 Conventional Treatment

Oral antibiotic treatment is the standard means of controlling and treating most cases of TB. For drug-susceptible TB disease, the initial intensive phase consists of a minimum of three drugs administered concurrently to reduce the rapidly dividing bacillary load; a minimum of two drugs is used in the continuation phase aimed at sterilizing lesions containing fewer and slow-growing bacilli. The first-line therapy consists of Isoniazid (INH), Rifampicin(RIF), Pyrazinamide(PZA), Ethambutol (EMB) and Streptomycin(S). For latent TB, 9 months isoniazide regimen is followed. DOTS is the treatment followed in India, the regimen is as shown in

Table 1. In case of MDR-TB the second-line class of drugs is made use of along with 1 or more 1st line drug. Second line drugs include aminoglycoside, antibiotics, cycloserine, ethionamide and fluoroquinolones. Second-line anti-TB drugs are employed only if the patient is not responding to the first-line therapy and/or are believed to be infected with drug-resistant strains of Mtb. Second-line agents are less effective and more toxic than the first-line drugs.[4]

1.3 Problems Associated with Conventional Oral Therapy

Table-1 : Treatment Regimen followed in India under Revised National Tuberculosis Control Programme (RNTCP), 1997

TB Category	Initial phase	Continuation phase	Total duration in months
New cases of smear positive pulmonary TB, severe extrapulmonary TB	$2H_3R_3Z_3E_3$	$4H_3R_3$	6
Failure, relapse TB cases	$2H_3R_3Z_3E_3S_3$ + $1H_3R_3Z_3E_3$	$5H_1R_1E_1$	8
New cases of smear negative cases	$2H_3R_3Z_3$	$4H_3R_3$	6

Explanation of Standard Code
1. Each anti-TB drug has a standard abbreviation (H-isoniazide,R-rifampin,Z-pyrazinamide, E-ethambutol, S-steptomycin)
2. Numeralcode before a phase is duration of that phase in month
3. The numeral in subscript is number of doses per week

Ref; K.D.Tripathi, Essentials of Medical Pharmacology, 5th edition, p: 706

Current TB therapy administered by the conventional routes, predominantly oral ingestion, in patients infected with drug-susceptible mycobacterial strains consist of a minimum of six to nine months of treatment regimen. Therapeutic drug concentrations are achieved in regions of the body having adequate blood circulation but poorly vascularized lesions, granulomas or tubercles, in the lungs harbor bacilli in microenvironments where hypoxic conditions confound treatment and extend therapy for many more months to be effective. Mycobacteria are protected in granulomas and conventional therapy may not penetrate into them at therapeutic levels. The standard therapy can be extended for up to two or more years if the patient fails to respond to the initial treatment based on sputum conversion or if patients are believed to be infected with drug-resistant strains. Mycobacterial strains exhibiting resistance to one or more drugs are arising at an alarming rate, requiring incorporation of more drugs in the increasingly complex combination therapy and consideration of extension of the duration of therapy. For oral delivery doses are higher due to low bioavailability resulting in more toxic effects. Prolongation of therapy may lead to reduced patient compliance and further increases the chances of emergence of drug-resistant strain. Intrapulmonary concentration of oral INH was below the MIC for mycobacterial strains in AIDS patients and normal subjects and may explain the rapid emergence of INH-resistant organisms when used alone to treat TB.[5]

To overcome these problems, search should be made for new drug molecules or modifying the drug delivery systems. Unfortunately drugs or vaccines have not been developed to rapidly prevent transmission to uninfected individuals or to treat apparently healthy, recently infected individuals. Over the past 45 years, new drug classes to treat TB have not been commercialized. Lung delivery as an adjunct to conventional therapy has the potential to decrease the dosing frequency and the duration of therapy.

2. Lung Delivery

2.1 Lungs Physiology

The human lungs are composed of a series of sub-dividing airways

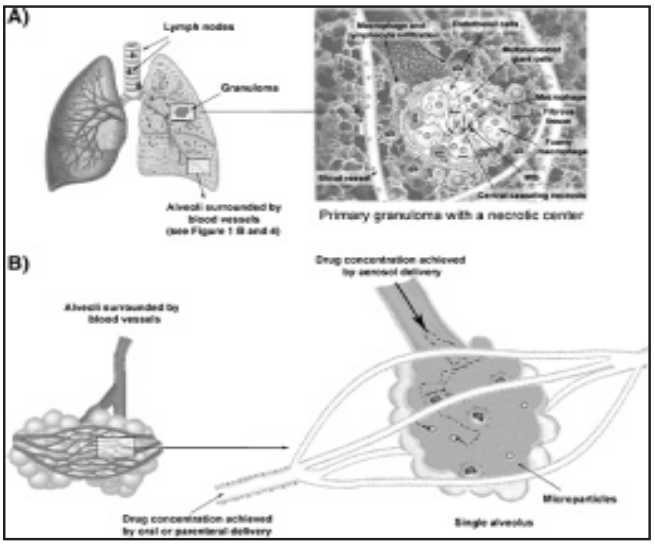


Fig. Microparticulate drug delivery to the lung for TB therapy.
A. The location and morphology of a granuloma in a diseased lung.
B. Lung alveoli surrounded by blood vessels
Ref : L Pharmaceutical Research, Vol. 26, No. 11, November 2009 .

with airway diameter diminishing from the trachea through the bronchi and bronchioles (known in total as the airways) and finally to the alveolar ducts and sacs, the sites of gas exchange. The pulmonary epithelium is thick (50–60 μm) in the trachea and poses a barrier to absorption. Towards the lower airways, the epithelium of the lung diminishes to a thickness of 0.2 μm in the alveoli .It is in this region that gas exchange occurs and the vast surface area of the alveoli (43–102 m^2 in an adult human) provides a highly vascularized expanse with access to the entire systemic circulation. The alveoli are in turn protected by alveolar macrophages, cells of the immune system that scavenge for foreign material along the lung surface, although particles too large or too small might be able to escape phagocytosis. Other immune cells such as dendritic cells also appear throughout the airways, where they sample for pathogens and foreign substances.[3]

2.2 Fate of inhaled particles

There are three principal mechanisms that lead to pulmonary

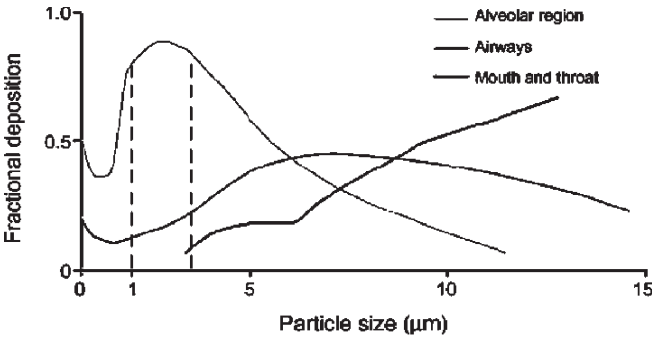
deposition-inertial impaction, gravitational sedimentation and Brownian diffusion. Where the particles deposit is characterized by their aerodynamic diameter, d_a which can be conceptualized by considering a spherical particle settling under gravity through air, the diameter of this spherical particle with density of 1 g/cm3 that has the same settling velocity as the particle of interest is the aerodynamic diameter.[3] This is defined by the equation:

Where
ρ = the mass density of the particle,
= the unit density (1 g/cm3) and
= the geometric diameter.

Large particles (> 5 μm) deposit by inertial impaction in the mouth and upper airways, smaller particles (= 1-5μm) deposit deeper in the lungs by inertial impaction and sedimentation, whereas very small particles (< 1μm) are driven by diffusion, mostly remain suspended and are exhaled. Particles less than 100 nm in diameter are able to deposit by diffusion in the alveolar region of the lungs, however, particles of this size are impractical drug delivery systems because large amounts of energy is required for either their creation (as liquid droplets) or their deaggregation (in dry powder form). Particles with aerodynamic diameter of 1 to 3 μm have been shown to deposit optimally in the alveolar region of the lungs.[7]

2.3 Modes of Respiratory Drug Delivery

A convenient way of delivering drugs to the lungs is the aerosolization



2. The effect of particle size on the deposition of aerosol particles in the human respiratory tract following a slow inhalation and a 5 s breath hold. Larger particles deposit in the airways or mouth and throat, whereas smaller particles deposit in the alveolar region. Particles <1μm can be exhaled, thereby reducing deep lung deposition.
Ref: W.Yang et al., International Journal of Pharmaceutics 356,2008, 239-247

of the drugs as fine powders with the aid of dry powder inhalers (DPIs). Alternatively, the drug may be first solubilized/suspended in an aqueous medium and subsequently aerosolized (liquid aerosolization or nebulization) through a nebulizer. A nebulizer requires a dispersing force (either a jet of gas or ultrasonic waves) for aerosolization.[8] A drug may also be delivered to the lungs directly, i.e. without prior aerosolization, using a device called an insufflators. Compared with a nebulizer, a DPI is more efficient in terms of drug delivery and less time consuming.[9] However, nebulizers can be designed to make the best use of a patient's breathing pattern, the so-called 'breath-assisted nebulizers'.[10] Further with jet nebulizers, adjustments in drug dosing are easier to achieve.[11]

3. Types of Lung Delivery Systems

3.1. Liposomes

Liposome-encapsulated drugs are especially effective against intracellular pathogens. They are one of the most extensively investigated systems for controlled delivery of drug to the lung, since they can be prepared with phospholipids endogenous to the lung as surfactants.[12]

3.1.1 Advantages:[13]

(a) The ability to formulate biologically active molecules.
(b) The ability to encapsulate both hydrophilic and lipophilic drug.
(c) Reduction in toxicity of the active agent.
(d) Increased therapeutic index.
(e) Increased stability of labile drugs.
(f) Improved pharmacokinetics.
(g) Increased delivery to target tissues.
(h) The feasibility of nebulization..

3.1.3. Special Features

Drug delivery systems using liposome-encapsulated antibiotics could improve antimicrobial activities against drug-resistant strains, because liposomes protect drugs by isolating them from degrading enzymes or promoting their diffusion across the bacterial envelope. Liposomes are easily captured by macrophages and neutrophils, and they accumulate in the organs of reticuloendothelial systems. Liposome-encapsulated antibiotics may be very useful against infections by intracytoplasmic pathogens. Liposomes are more popular as intravenous Anti tubercular drugs (ATD) carriers. However, liposomes have been successfully nebulized to treat intracellular pulmonary infections. Conventional (phosphatidylcholine/ cholesterol) liposomes encapsulating rifampicin and isoniazid are prepared for nebulization.[14]

Vyas et.al developed RIF-containing aerosolized micrometric liposomes to target the alveolar macrophages, which is a prevalent infection site. They anchored alveolar macrophage-specific ligands such as Maleylated Bovine Serum Albumin (MBSA) and O-steroyl amylopectin (OSA) to the surface of the nanocarriers with the intention to improve the selectivity for the lung. MBSA is recognized by macrophage scavenger receptors and OSA shows affinity for alveolar macrophages. Liposomes were formulated in chlorofluorocarbon propellants and packed in a pressurized container.[15]

Nebulization of liposomal ATDs, coupled to the use of alveolar macrophage-specific ligands may improve the chemotherapy of pulmonary TB especially in view of the fact that liposomes are known to be safe when administered via the respiratory route.

3.2. Microparticles

Polymeric microparticles produced from natural and synthetic polymers have been extensively investigated as drug carriers for administration via a number of different routes.[16] The various drug carriers used are poly (lactide-co-glycolide) (PLG), polylactic acid (PLA) and dipalmitoyl glycerophosphocholine (DPP).[17]

3.2.1 Advantages

1. Providing sustained drug release.
2. Micro particulate preparations can target AMs that harbor the TB bacteria in pulmonary TB.
3. In the case of pulmonary TB, delivering the drug directly to the site of infection as inhalation aerosols can also bypass the first-pass metabolism and maintain local therapeutically effective concentration with decreased systemic side effects.
4. It can be prepared over a wide range of particle sizes, which is a decisive factor in the in vivo disposition of particulate carriers.

PLG microparticles encapsulating rifampicin were prepared by employing solvent evaporation as well as spray drying methods. The former technique resulted in spherical particles with 20% drug loading and 3.45 μm volume median diameter whereas the latter technique produced shrivelled particles with 30% drug loading and 2.76 ìm diameter. The microspheres were administered via insufflation or nebulization to guinea pigs. The assessment of colony forming units (cfu) 28 days post-infection showed a dose–effect relationship, i.e. lower cfu with higher doses of microspheres. The cfu count was significantly reduced compared with free rifampicin.[18]

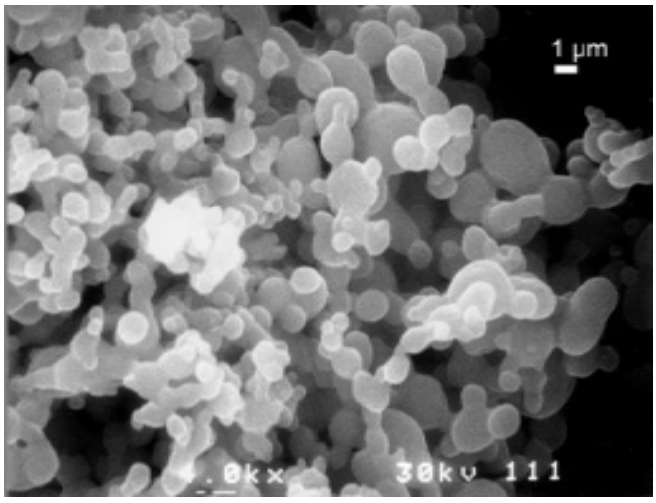


Fig. 1 : Representative SEM of drug-loaded PLA microparticles produced by PCA processing. Shown here in the picture are PLA microparticles loaded with TPA-INHMS complexes (magnification_4600). Scale bar=1 Am

Ref: H. Zhou, Journal of Controlled Release, 292, 107, 2005, 288–299

3.3 Nanoparticles

Nanoparticles delivery to the lungs is an attractive concept because it can cause retention of the particles in the lungs accompanied with a prolonged drug release if large porous nanoparticles matrices are used. On the other hand studies have shown that nanoparticles uptake by alveolar macrophages can be reduced if the particles are smaller than 260 nm. Both effects combined might improve local pulmonary drug therapy. However, the particle size of medically used nanoparticles is too small to be suitable for direct lung delivery. A prerequisite for deep lung delivery is the design of proper carrier systems. Successful delivery of inhaled particles depends mostly on particle size and particle density and hence, the mass median aerodynamic diameter. The respirable fraction of an inhalable powder is generally the fraction of particles with an aerodynamic diameter ranging between 1 and 5 μm. This size range guarantees a maximum deposition in the deep lung.[19] Formulation systems includes materials like PLGA (poly(lactide-co-glycolide) PVA,WGA lectin, alginates etc. PLG is the most intensively studied nanoparticulate drug carrier.[20]

3.3.1 Technological Advantages of Nanoparticles as Drug Carriers:[21]

1. High stability (i.e. long shelf life)
2. High carrier capacity (i.e. many drug molecules can be incorporated in the particle matrix)
3. Feasibility of incorporation of both hydrophilic and hydrophobic substances
4. Feasibility of variable routes of administration, including oral administration and inhalation.
5. These carriers can also be designed to enable controlled (sustained) drug release from the matrix, resulting in a reduction in dosage frequency and improved patient compliance
6. Nanoparticles with their special characteristics such as small particle size, large surface area and the capability of changing their surface properties have numerous advantages compared with other delivery systems.

Hence nanoparticles are a very promising drug delivery system via different routes of administration in the treatment of pulmonary tuberculosis.

3.4 Solid-Lipid Nanoparticles

A new concept in nanotechnology is that of Solid-Lipid Nanoparticles (SLNs), i.e. lipid nanocrystals in water possessing a solid core into which drugs are incorporated.

3.4.1 Advantages

The SLNs combine the virtues of more traditional drug carriers such as liposomes or polymeric nanoparticles while eliminating some of their disadvantages, e.g. the issues of burst release and long-term stability in the case of liposomes as well as the problems of residual solvents and bulk production in the case of polymeric nanoparticles. Furthermore, although PLG is completely biodegradable and biocompatible, the degradation rate is slow and repeated administration of the formulation carries a likelihood of accumulation of the polymer or its degradation products in the respiratory tract. The polymer is known to elicit a mild inflammatory response lasting 2–3 weeks.[22,23]

4. Advantages of Pulmonary Drug Delivery.[24]

1. The lungs have high solute permeability.
2. Large surface area for absorption.
3. Limited proteolytic activity.
4. Pulmonary delivery is non-invasive.
5. Pulmonary delivery can potentially result in reducing the overall dose and the amount of side effects that result from high levels of systemic drug exposure.
6. It is patient friendly since administration of drug is easy & frequency of dose administration is low compared to oral route.

Properties of Important Drug Carriers

Carrier	Features
Liposomes	Made up of physiological lipids low encapsulation of polar drugs targetable to macrophages problems of fluctuation of drug and short half-life
Microparticles	High drug encapsulation for hydrophilic & lipophilic drugs good pulmonary deposition poor systemic bioavailability following inhalation.
Nanoparticles	High drug encapsulation good pulmonary as well as systemic bioavailability
Solid Lipid Nanoparticles	Natural carrier based high drug encapsulation good pulmonary deposition as well as systemic bioavailability long shelf-life

Ref : Pharmaceutical Research,Volume 26, No.11, November 2009.

7. Dose compliance is very good since doses are reduced since higher concentrations of drugs can be achieved in lungs.

Following table gives the comparative results of above systems and shows the salient features of inhalable Anti-Tubercular Drug delivery systems.

5. Advances in Pulmonary Delivery

5.1 A novel particulate form incorporating nanoparticles into micron-scale structures has been engineered to overcome the issues of storing and delivering nanoparticles to the lungs. It is referred to as 'porous nanoparticles-aggregate particles' (PNAPs). These structures embrace the virtues of both nano and micron-scale particles. By formulating the nanoparticles into larger hollow or porous structures, the dry powders can be delivered more easily into the lungs with a simple inhaler. The matrix of the carrier micro particle can consist of only nanoparticles or additional inert pharmaceutical excipients, such as amino acids, sugars or phospholipids. Upon deposition in the lungs and exposure to the humid environment and the lung lining fluid, the matrix of the PNAPs dissolves and readily releases the nanoparticles.[25]

5.2. Administration of BCG vaccine for pulmonary TB through nasal route is also being investigated. There is also increasing experimental evidence in mice that nasal vaccination is the best way to attain robust immune responses in the lungs.[26]

6. Conclusion

Though the oral therapy for pulmonary tuberculosis is effective,

Delivery system	Mode of delivery	Drug loading	Respirable amount	Mass median aerodynamic diameter	Animal model	Retention in Pharynx	Retention in Lungs
1. Liposomes							
(a) Conventional	Nebulization	Rifampicin 22% Isoniazid 14%	94%	0.96 μm	Guinea pigs	2 days	5 days
(b) Lipid-suspended	Nebulization	Rifampicin, 40%	NR	NR	Rats	NR	> 1 day
2. Microparticles							
(a) PLG	Nebulization/ Insufflation	Rifampicin, 20%	NR	2.76 μm	Guinea pigs	< 3 days	3 days
(b) PLA	Dry powder inhalation	Rifampicin, 11% Isoniazid, 4%	32%	6.20 μm	Rats	NR	NR
(c) DPP	Insufflation	BAS, 95%	62%	7.07 μm	Rats	< 3 h	> 3 h
3. Nanoparticles							
a) PLG	Nebulization	Rifampicin Isoniazid Pyrazinamid	60–70%	1.88 μm	Guinea pigs	6–8 days	9–11 days
b) Lactide-PLG	Nebulization	Rifampicin Isoniazid Pyrazinamide	60–70%	2.80 μm	Guinea pigs	6–14 days	15 days
4. Solid Lipid Nanoparticles	Nebulization	Rifampicin Isoniazid Pyrazinamide	40–50%	1.80 m	Guinea pigs	5 days	7 days

PLG, poly(lactide-co-glycolide); PLA, polylactic acid; DPP, dipalmitoylphosphatidylcholine; BAS, para-aminosalicylic acid; NR, not reported

Ref : Pharmaceutical Research, Vol. 26, No. 11, November 2009 (# 2009)

the dose, duration and side effects can greatly be reduced by using direct delivery to lungs. Recent advances in aerosol technology are helpful for development of various pulmonary drug delivery systems. This system can concurrently be used along with oral treatment or it can totally replace the oral treatment.

References

1. World Health Organization (WHO).Tuberculosis Fact sheet No104—Global and regional incidence, March 2009.
2. Haas DW. Principles and practice of infectious diseases, Philadelphia: Churchil Livingstone; 2000. 2576-2607.
3. Tortora W., Principles of anatomy and physiology, 10th edition.
4. Tripathi K. D., Essentials of medical pharmacology, 6th edition
5. Conte JE, McKenna E, Effects of gender, AIDS, and acetylator status on intrapulmonary concentrations of isoniazid, Antimicrob Agents Chemother. 2002;volume 46(8):2358-64.
6. Hinds W.C, Aerosol technology: properties, behavior and measurement of airborne particles (2nd edn), Wiley, 2000.
7. Heyder J. Deposition of particles in the human respiratory tract in the size range 0.005–15 mm. Journal of Aerosol Science, 17, 1989, 811–825
8. O'Riorden, T. G. Formulations and nebulizer performance. Respiratory Care 47, 1305–12.
9. Le Brun, P. P., de Boer, A. H., Heijerman, H. G. A review of the technical aspects of drug nebulization, Pharmacy World and Science,22,2000, 75–81.
10. O'Callaghan, C. & Barry, P. W. The science of nebulized drug delivery. Thorax 52, Suppl. 2, 1997, S31–S44.
11. Rau J.L. Design principles of liquid nebulization devices currently in use. Respiratory Care 47,2002, 1257–1275.
12. Shigeru, Kazunori, and Shigefumi, Drug delivery systems for infection: liposome-incorporating antimicrobial drugs, Review Article J Infect Chemother, 4.1998,159-173.
13. Khuller G.K, Kapur M & Sharma S, Liposome technology for drug delivery against mycobacterial infections. Current Pharmaceutical Design 10, 2004, 3263–74.
14. Bermudez L.E., Use of liposome preparation to treat mycobacterial infections, Immunobiology 191, 1994, 578–583.
15. Vyas, S. P, Kannan, M. E, Jain S. Design of liposomal aerosol for improved delivery of rifampicin to alveolar macrophages. International Journal of Pharmaceutics 269,2004, 37–49.
16. Pandey, R. & Khuller, G. K. Polymer based drug delivery systems for mycobacterial infections. Current Drug Delivery 1,2004, 195–201.
17. Jain R.A. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21,2000, 2475–90.
18. Suarez S, O'Hara P, Kazantseva, M. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: screening in an infectious disease model. Pharmaceutical Research 18, 2001, 1315–1319.
19. Yanga W., Petersb J.I., Williams R.O. III, Inhaled nanoparticles—A current review International Journal of Pharmaceutics 356 2008, 239–247.
20. Rajesh Pandey and G. K. Khuller, Antitubercular inhaled therapy: opportunities, progress and challenges, Journal of Antimicrobial Chemotherapy 55, 2005, 430–435.
21. Pandey, R. & Khuller, G. KPolymer based drug delivery systems for mycobacterial infections. Current Drug Delivery 1, 2004, 195–201.
22. Rao G.C, Kumar M. S, Mathivanan N. Nanosuspensions as the most promising approach in nanoparticulate drug delivery systems, Pharmazie 59,2004, 5–9.
23. Dingler A. & Gohla S, Production of solid lipid nanoparticles (SLN): scaling up feasibilities. Journal of Microencapsulation 19,2002, 11–6.
24. Patton, J.S. Mechanisms of macromolecule absorption by the lungs. Adv. Drug. Deliv. Rev. 19,1996, 3–36
25. Review of TRENDS in Biotechnology 25,No.12.
26. lleniusa G., Pawlowskia A., Brandtzaegc P., Svensona S., Should a new tuberculosis vaccine be administered intranasally? Tuberculosis 87, 2007, 257–266.