Chapter Two

Literature Review
2. LITERATURE REVIEW

The present work deals with the formulation of an oral sustained release drug delivery system of isoniazid and rifampicin, with the aim to reduce the extent of drug interaction between the two drugs. Various drug delivery systems used in earlier studies and the exploitation of microparticulate technology for oral route of administration have been discussed.

2.1. Studies on Interaction between Isoniazid and Rifampicin

Singh et al., (2000) carried out a study to determine the extent of degradation of isoniazid, pyrazinamide, and rifampicin from prepared mixtures and marketed preparations containing single, two, and four drugs, under stomach conditions. The results of comparative study showed that rifampicin was decomposed by 17.8-24.4%, isoniazid to a lesser extent (3.2-4.7%) and pyrazinamide was stable. The decomposition of rifampicin was influenced by isoniazid but not by pyrazinamide or ethambutol.

Shishoo et al., (2001) studied the comparative bioavailability of rifampicin after administration of a single component rifampicin capsule and rifampicin, isoniazid FDC capsule formulation in healthy male volunteers. HPTLC method was used to estimate the drug and its major active metabolite, 25-Desaetylrifampicin levels in urine. The study confirmed the doubt about the stability of rifampicin in presence of isoniazid in acidic environment of stomach resulting in reduced bioavailability of rifampicin.

Singh et al., (2002) carried out an investigation to determine the behavior of moisture gained by antitubercular agents when exposed in pure form and in combination to accelerated conditions of 40 °C and 75 % relative humidity, in absence and presence of light.

Singh and Mohan (2003) carried out a pilot stability study on four FDC antitubercular products at 40 °C and 75 % relative humidity. The products in unpacked condition showed sever decomposition of rifampicin and extensive physical changes. The main decomposition product in the solid state was isonicotinyle hydrazone of 3-formyl rifampicin and isiniazid.
2.2. Reported Formulations of Antitubercular Agents

2.2.1. Parenteral drug delivery

2.2.1.1. Liposomes

Gaspar et al., (2000) developed liposomal formulation of rifabutin and the effects of some parameters on the incorporation efficiency were studied. The activity of rifabutin incorporated in to liposomes prepared with phosphatidylcholine and phosphatidylserine (7:3) was evaluated in a murin model of infection with avirulent *M. avium* strain (strain P1581) and was compared with that of free rifabutin. These results demonstrate that liposomal formulations of antibiotics such as rifabutin could be effective for the treatment or prophylaxis of infectious diseases.

Labana et al., (2002) studied the chemotherapeutic potential of isoniazid and rifampicin encapsulated lung specific stealth liposomes at one third of their recommended doses. The formulation showed sustained release of these drugs in plasma (5 days) and lung, liver and spleen (7 days). Administration of liposomal formulation for 6 weeks reduced the mycobacterial load significantly in lung, liver, spleen of infected mice compared with treated animals.

Das et al., (2003) described the development of a novel thiocationic lipid-based formulation of phosphothionate antisense oligonucleotides showing inhibitory activity against *M. tuberculosis* as measured by an *in vitro* BACTEC 460 TB assay. Liposomal formulations resulted in statistically significant inhibition compared with thiocationic liposomal control and liposomal components.

Maurizio Ricci et al., (2006) developed capriomycin sulfate liposomes. They studied the effect of formulation variables on peptide encapsulation by using a $2^3$ factorial design.

2.2.1.2. Niosomes

Jain and Vyas (1995) prepared niosomes containing rifampicin of 8-15 μm diameter using Span 80 and cholesterol in various molar fractions. The process variables that could affect the physical characteristics of niosomes and *in vitro* release of the drug from the niosomes were...
studied and optimized. In vivo distribution studies of the prepared niosomes found that 65% of the drug could be localized in the lungs by controlling the noisome size.

Kamath et al., (2000) designed liposome and niosome-encapsulated drug delivery systems for rifampicin and evaluated the same in vitro and in vivo. A modified lipid layer hydration technique was employed to prepare these vesicular carriers. Both the product exhibited the sustained release characteristics in vitro with zero order drug release kinetics up to 10 h.

2.2.1.3. Microparticles

Quenelle et al., (1999) prepared microsphere formulations for targeted and sustained delivery of rifampicin, with minimal dosing of lactide and glycolide copolymers. A small-microsphere formulation, with demonstrated ability to inhibit intracellularly replicating M. tuberculosi s H37Rv, was tested along with a large-microsphere formulation in an infected mouse model. The microsphere formulation, administered in one or two dosage, were able to achieve results in mice similar to those obtained with daily drug regimen within the range of the highest clinically tolerated dosage in humans.

Zhang et al., (2000) prepared rifampicin polyactic acid microspheres for lung targeting by a modified emulsion solvent diffusion method. Drug content, particle size distribution and in vitro release properties of the prepared microspheres were evaluated. In vivo experiments on rabbit showed remarkable accumulation of microspheres in lung.

Dutt and Khullar (2001) developed PLG microspheres as carriers for isoniazid and rifampicin in order to improve patient compliance of tuberculous chemotherapy. Antitubercular drugs encapsulated in PLG polymers and injected subcutaneously resulted in a sustained release (up to 6 weeks) of drugs in various organs of mice.

Quenelle et al., (2001) reported the use of rifampicin-loaded microspheres to effectively treat M. tuberculosis infected macrophages and mice using lactide and glycolide copolymers. The formulations were evaluated individually and in combination with oral regimens of isoniazid. Treatment with rifampicin-loaded microspheres alone resulted in significant reduction in the number of colony forming unit (CFU) in lung and spleen by 26 days. Combination therapy of isoniazid with rifampicin-loaded microspheres increased the effective range. In many cases,
complete elimination of CFU was obtained with the combination therapy, something not achieved with most of the single therapies.

Lucinda-Silva and Evangelista (2003) prepared microspheres of isoniazid-alginate-chitosan by means of a complex coacervation method in an emulsion system. Since the encapsulation of isoniazid tends to be limited due to its hydrophilic characteristics, this study proposes its encapsulation by adsorption. The particles were prepared in three steps: (a) preparation of a w/o emulsion (b) phase separation; and (c) adsorption of drug. The adsorption observed is probably of chemical nature, i.e. there is an ionic interaction between the drug and the surface of the particles.

2.2.1.4. Nanoparticles

Lopes et al., (2000) evaluated the association of ethionamide with different colloidal systems. Nanocapsules, nanospheres and nanoemulsion were prepared by interfacial deposition and spontaneous emulsification techniques. The release profile demonstrated that associated ethionamide was more readily released from nanocapsules and nanospheres. The drug was mainly adsorbed on to the surface of the nanoparticles. However, approximately 10% of ethionamide was encapsulated into nanocapsules and 20% entrapped in to nanospheres respectively.

Skidan et al., (2003) conducted studies that revealed association of rifampicin with polybutylcyanoacrylate nanoparticles provided considerable enhancement of the drug’s antibacterial activity. In vitro nanoparticle loaded rifampicin was more active against Staphylococcus aureus and M. avium localized in isolated alveolar macrophages. Single administration of rifampicin loaded nanoparticles in the dose 25 mg/kg resulted in 80% survival of mice, while 50 mg/kg of free rifampicin could provide only 10% survival.

Pandey and Khullar (2004a) prepared PLG nanoparticles encapsulating isoniazid, pyrazinamide and rifampicin by multiple emulsion technique. A single subcutaneous dose of drug loaded PLG nanoparticles resulted in sustained therapeutic drug levels in the plasma for 32 days and in the lung/spleen for 36 days.
2.3. Inhalational Drug Delivery

2.3.1. Liposomes

Vyas et al., (2004) formulated and evaluated rifampicin loaded aerolized liposomes for their selective presentation to alveolar macrophage that being the densest site tuberculosis infection. In vitro drug release was recorded 1.5-1.8 times higher as compared to plain drug solution-based aerosol. Percent viability of M. smegmatis inside macrophages after administration of drug was 7-11 % in case of ligand anchorliposomal aerosol and 45.7 % and 31.6 % in case of plain drug and plain neutral liposomal aerosol treated macrophages. These results suggested that the ligand anchor liposomal aerosols were not only effective in rapid attainment of high-drug concentration in lung with high population of alveolar macrophages but also maintain the same over prolonged period of time.

2.3.2. Microspheres

Sharma et al., (2001) designed inhalable biodegradable poly (DL-lactic acid) microparticles containing isoniaid and rifampicin and tested them for activity against the tuberculosis. Inhalable microparticles containing multiple antitubercular drugs showed promising results.

Suarez et al., (2001) used M. tuberculosis (H37Rv) - infected guinea pig model to screen for targeted delivery to the lungs by insufflation (with lactose excipients) or nebulization, of either rifampicin alone, rifampicin with PLG microspheres or PLG microspheres alone. Animal treated with single and double doses of rifampicin-PLG microspheres exhibited significantly reduced numbers of viable bacteria, inflammation and lung damage. Two doses of rifampicin-PLG microspheres resulted in reduced splenic enlargement.

Sethuraman and Hickey (2002) determined aerosol delivery of rifampicin loaded microparticles to lungs infected with M. tuberculosis could be achieved by predicting dispersion of dry powder through knowledge of particle surface properties. Various processing factors were studied and their effect was assessed on the formulation.

Tsapis et al., (2003) formulated large porous particles of para-aminosalicylic acid (PAS) for direct delivery into the lung via inhalation. These results suggested that inhalation delivery of PAS could potentially allow for a reduction in total dose delivered while providing for higher
local and similar peak systemic drug concentrations as compared to those obtained upon oral PAS dosing.

2.3.3. Nanoparticles

Pandey et al., (2003a) reported the formulation of rifampicin, isoniazid and pyrazinamide encapsulated in PLG nanoparticles suitable for nebulization. A single nebulization to guinea pigs resulted in sustained therapeutic drug levels in the plasma for 6-8 days and in the lungs for up to 11 days. On nebulization of nanoparticles containing drugs to *M. tuberculosis* infected guinea pigs at every $10^3$ day; no tubercle bacilli could be detected in the lung after five doses of treatment.

2.4. Oral Drug Delivery

2.4.1. Microparticles

Dutt and khullar (2000) investigated PLG as carrier for rifampicin. Different formulations of PLG microparticles viz. porous, non-porous and hardened exhibited sustained release of rifampicin up to 7 weeks *in vitro*. However, hardened PLG microparticles exhibited the most sustained release *in vivo* in different group up to 6 weeks.

Dutt and khullar (2001) developed PLG microparticles containing a combination of rifampicin and isoniazid sustained release carrier systems. A single dose of microparticles exhibited a sustained release of rifampicin and isoniazid *in vivo* up to 7 and 6 weeks respectively.

Ain et al., (2002) developed an oral formulation based on PLG microparticles for delivery of antitubercular drugs. This formulation was found to be stable in the acidic environment of gastric fluid whereas, in the intestinal fluid the drug release was obtained up to 20 days as indicated by *in vivo* studies.

Ain et al., (2003) developed alginate microparticles as oral sustained delivery carriers for antitubercular drugs in order to improve patient compliance. These microparticles exhibited sustained release of rifampicin, pyrazinamide and isoniazid for 3-5 days in plasma and up to 9
days in organs. Chemotherapeutic efficacy of microparticles against experimental tuberculosis showed no detectable CFU. Histopathological observation were also supported this study.

Pandey and Khullar et al., (2004b) developed alginate-chitosan microspheres as drug carriers to reduce dose/dosing frequency in the management of tuberculosis. A therapeutic dose and a half-therapeutic dose of the microsphere-encapsulated drugs were orally administered to guinea pigs for pharmacokinetic/chemotherapeutic evaluations, respectively. In *M. tuberculosis* H37Rv-infected guinea pigs, administration of a therapeutic dose of microspheres spaced 10 days apart produced a clearance of bacilli equivalent to conventional treatment for 6 weeks.

2.4.2. Nanoparticles

Pandey et al., (2003b) reported the formulation of rifampicin, pyrazinamide and isoniazid encapsulated in PLG nanoparticles. Following a single oral administration of these preparations to mice, the drugs could be detected in the circulation for 6 days (rifampicin) and 9 days (isoniazin/pyrazinamide), whereas therapeutic concentrations in the tissues were maintained for 9-11 days. Further, on oral administration of drug loaded nanoparticles to *M. tuberculosis*-infected mice at every 10th day; no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment.