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Formulation of an anti-tuberculosis drug delivery system

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CHAPTER 1

Introduction

1.1. Tuberculosis – Delineating the Disease

Tuberculosis (TB), a ubiquitous, highly contagious chronic granulomatous bacterial infection, is still a leading killer of young adults worldwide. TB has returned with a new face and the global scourge of multi-drug resistant TB (MDR TB) is reaching epidemic proportions, not to mention the recent reports of the burgeoning spread of extreme drug resistant strains.

Nearly one-third of the global population - two billion people - is infected with *Mycobacterium tuberculosis (M. tuberculosis),* more than eight million people develop active TB every year, and approximately two million die annually (World Health Organization, 2003). TB is the world's second most common cause of death from infectious disease, after acquired immunodeficiency syndrome (AIDS) (Frieden, 2003). It is endemic in most developing countries and resurgent in developed and developing countries with high rates of human immunodeficiency virus (HIV) infection. With particular reference to Africa the increase in TB incidence is strongly associated with the prevalence of HIV infection: rates of HIV infection among TB patients are correspondingly high, exceeding 60% in South Africa, Botswana, Zambia, and Zimbabwe (Singh, 2004).

Mortality rates of TB range from 50 to 80% in untreated smear-positive individuals to 30% with inconsistent control programmes and drop to lower than 5% when directly observed therapy (DOT) and active TB control programmes are instituted (Singh, 2004).

The TB incidence (number of new cases arising each year) and mortality in each of the WHO regions is depicted in Figure 1.1. The incidence of all forms of TB, the incidence of infectious

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cases, and mortality are represented as the rate per 100 000 population (WHO, 2003). The majority of cases (5–6 million) are in people aged 15–49 years. The largest number of cases occurs in the South-East Asia Region, which accounts for 33% of incident cases globally. However in 2003, the estimated incidence per capita in sub-saharan Africa was nearly twice that of the South-East Asia, at 290 to 350 cases per 100 000 population (Frieden, 2003; WHO, 2003).

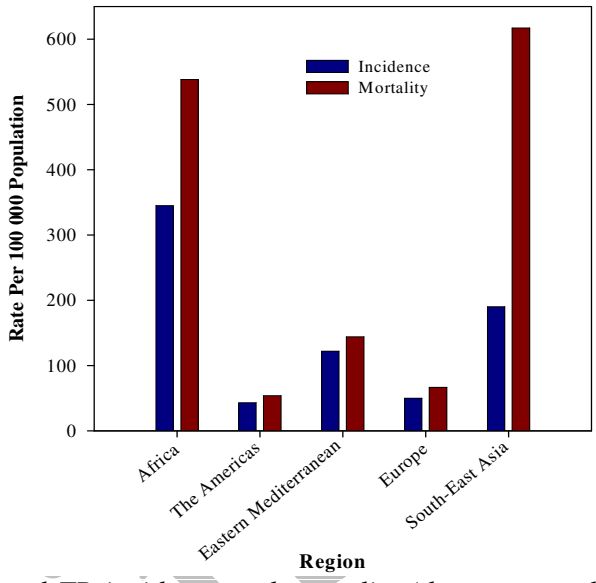

Figure 1.1: Estimated TB incidence and mortality (data extracted from WHO Tuberculosis data sheet, 2003; WHO Global Tuberculosis Database, 2004, as of latest available worldwide data, March 2006)

M. tuberculosis is a highly contagious, airborne, slow-growing, Gram-positive aerobic rod-shaped acid-fast bacillus. The cell wall has high lipid content and allows the bacteria to survive within macrophages. It also provides the organism with a resistant barrier to many common drugs (Katzung, 2002; Williams and Lemke; 2002). *M. tuberculosis* virulence factors have been the subject of recent intense exploration. Three major virulence factors from the outer layer of the mycobacterial cell wall have been molecularly characterised: *M. tuberculosis* cord factor, mycobacterial sulpholipids and mycosides; which are trehalose-6,6'-dimycolates, trehalose-2'-sulphates and species-specific glycolipids and peptidoglycolipids of mycobacteria, respectively (Friedman, 2001).

Man is the primary host for *M. tuberculosis.* Infection is spread via airborne dissemination of aerosolised bacteria-containing droplet nuclei of 1–5 μm in diameter that carry *M. tuberculosis* bacilli from an individual with infectious TB disease to an uninfected individual. The infectious droplet nuclei are inhaled and lodge in the alveoli in the distal airways. *M tuberculosis* is then taken up by alveolar macrophages, initiating a cascade of events that results in either successful containment of the infection or progression to active disease (primary progressive TB). During the time required to develop cell-mediated immunity to bacterial growth, *M. tuberculosis* infection can spread by lymphohaematogenous dissemination to other sites.

In the majority of infected individuals, development of cell-mediated immunity leads to either local destruction of *M. tuberculosis* or persistance of organisms in a latent phase within tissue macrophages, often for a lifetime. Foci with latent *M. tuberculosis* infection are sites of original dissemination and include the apices of the lungs, cortices of the kidneys, and growing ends of long bones - tissues having a high local oxygen concentration, which promotes *M. tuberculosis* growth within human macrophages. In 5-10% of cases due to failure of immunologic surveillance against *M. tuberculosis* infection, bacillary multiplication resumes and manifests as clinical TB.

Risk of development of active disease varies according to time since infection, age, and host immunity, however, the lifetime risk of disease for a newly infected young child has been estimated at 10% (Comstock et al., 1974; Sutherland, 1976; Frieden, 2003).

Granuloma formation with caseation in various tissues is the pathologic hallmark of primary and reactivation TB. Tuberculous granulomas are characterised by blood-derived macrophages with nuclei around the periphery of the giant cell (Langhan's type) and T

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lymphocytes around the periphery of the granuloma. Resultant liquefication of the caseous material may occur, with caseous necrosis and cavity formation resulting from sensitivity to *M. tuberculosis* proteins. It is purported that hydrolytic enzymes and oxygen radicals produced by macrophages and neutrophils mediate much of the pathological tissue damage, in addition to cytokines (e.g. interleukin-1, IL-1, and interleukin-6, IL-6, and tumour necrosis factor-α, TNF-α) produced by mononuclear cells at the sites of active *M. tuberculosis* infection (Friedman, 2001).

Here, it is of importance to stress the impact of TB and its pathogenesis on HIV infection, as they are undoubtedly inextricably linked. The significance of effective TB chemotherapy and compliance-promoting approaches is an underlying philosophy in developing novel anti-TB drug delivery systems. HIV-infected patients with TB have decreased survival and increased opportunistic infections and a greater decrease in CD4+ T-lymphocyte counts relative to CD4-matched controls. Development of TB is associated with an increase in plasma HIV ribonucleic acid. Additionally, blood monocytes from patients with TB release increased amounts of IL-1, IL-6, and TNF-α, which activate replication of HIV. HIV infection in latently infected cell lines and HIV-infected monocytes is also enhanced by mycobacteria and their protein and polysaccharide constituents. TB generates a cytokine microenvironment that actually enhances infection of lymphocytes by HIV (Friedman, 2001).

The current understanding of TB pathogenesis demonstrates varying levels of complexity and no single approach to the elucidation of TB pathogenesis encompasses its entirety. Recent progress in the understanding of *M. tuberculosis* has allowed a more complete view of how the pathogen interacts with the host (Friedman, 2001). Schematic representation of the progression of TB is shown in Figure 1.2.

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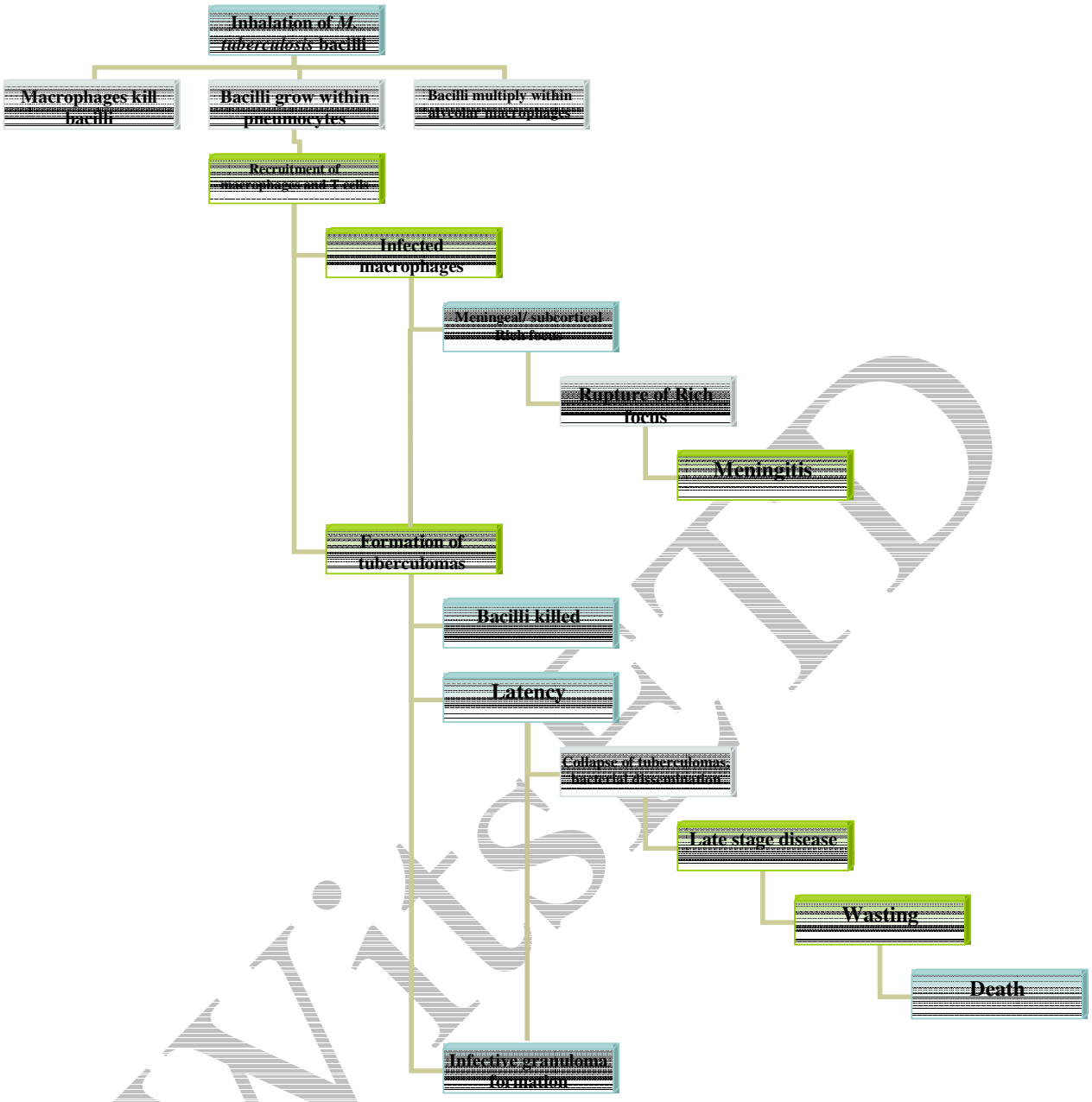

Figure 1.2: Pathogenesis of TB

1.2. Current Anti-tuberculosis Chemotherapy

Since the control measures for TB such as Bacillus Calmette-Guérin (BCG) vaccination and chemoprophylaxis appear to be unsatisfactory, treatment with anti-tubercular (anti-TB) drugs becomes the only option available. The goals of treatment are to ensure cure without relapse, to prevent death, to stop transmission, and to avert the emergence of drug resistance. Long-term treatment with a combination of drugs is still paramount for success (Fox et al., 1999).

Treatment of active TB with a single drug should never be attempted, and a single drug should on no account be added to a failing regimen, the result being development of MDR TB – simultaneous resistance to the frontline anti-TB drugs, RIF and INH (Iseman, 1993).

As suggested by WHO (1999), treatment of TB and drug resistant cases requires multi-drug therapy, comprising:

1. An initial intensive phase of rifampicin (3-[4-methylpiperazinyliminomethyl]-rifamycin SV) (RIF), isoniazid (isonicotinic acid hydrazide) (INH), pyrazinamide (pyrazinecarboxamide) (PYZ), and ethambutol (ETB) daily for 2 months.
2. A continuation phase of RIF and INH for a further 4 months, either daily or 3 times per week, to be administered as advised in Table 1.1.

Table 1.1: Regimen 1 – for treatment of new smear positive adult patients (Gibbon, 2004)

Intensive Phase – 2 months	**Under 50 kg**	**Over 50 kg**
RIF/INH/PYZ/ETB Combination tablet 120/60/300/200 mg daily, 5 days per week	4 tablets	5 tablets
Continuation phase – 4 months	**Under 50 kg**	**Over 50 kg**
RIF/INH Combination tablet 150/100mg	3 tablets	
Combination tablet 300/150 mg	-	2 tablets

INH eradicates most of the rapidly replicating bacilli in the first 2 weeks of treatment, together with streptomycin and ETB. Thereafter, RIF and PYZ have an important role in the sterilisation of lesions by eradicating organisms; these two drugs are crucial for successful 6-month treatment regimens. RIF kills low or non-replicating organisms and the high sterilising effect of PYZ serves to act on semidormant bacilli not affected by any other anti-TB agents in sites hostile to the penetration and action of the other drugs (Garg, 1999). INH and RIF, the two most potent anti-TB drugs, kill more than 99% of tubercule bacilli within 2 months of initiation of therapy (Mitchison, 1985; Iseman and Madsen, 1989). Using these drugs in conjunction with each other reduces anti-TB therapy from 18 months to 6 months. The sites

and speed of action of these principle anti-TB agents are schematically illustrated in Figure 1.3, as delineated by Rattan et al. (1998), Somoskovi et al. (2001), Mitchison (2005), and Parsons et al. (2005). Therefore, the emergence of strains resistant to either of these drugs causes major concern, as treatment is then deferred to drugs that are less effective, have more toxic side effects, and result in higher death rates, especially among HIV-infected persons (Rattan et al., 1998**).** Numerous authors have reviewed the current armamentarium of drugs available the treatment of TB, their mechanism of action, and activity (Table 1.2).

TB is treated with a multi-drug regimen, and is thus exceptionally vulnerable to incidences of side effects, unsatisfactory patient compliance and slow improvement of patients (Prabakaran, 2004). Therefore, despite the availability of these highly effective treatments for TB, cure rates remain low, as commercial anti-TB formulations are inconvenient to administer and patients do not take the prescribed medications with sufficient regularity and duration to achieve a cure (Chen, 2000). Patients have to consume an excessive number of tablets (i.e. in South Africa, up to 5 large combination tablets of 14.5mm diameter), which is a common cause for non-compliance. It can be anticipated that non-optimal application of these short course regimens will result in the deterioration of their therapeutic potential, an escalation in the mortality rate and increased risk of developing acquired drug resistance (Agrawal et al., 2002; WHO, 2003). Resistance of *M. tuberculosis* to anti-TB agents is a worldwide problem in both immunocompetent and HIV-infected populations (Edlin, 1992; Fischl, 1992).

As succinctly stated by Khuller and Pandey (2003), '*Patients often find it troublesome to begin their day with a mouthful of pills*'. Additionally, as the patients' clinical symptoms improve, they may not consider the need to continue with the anti-TB drug regimen and may actually forget to take their medication.

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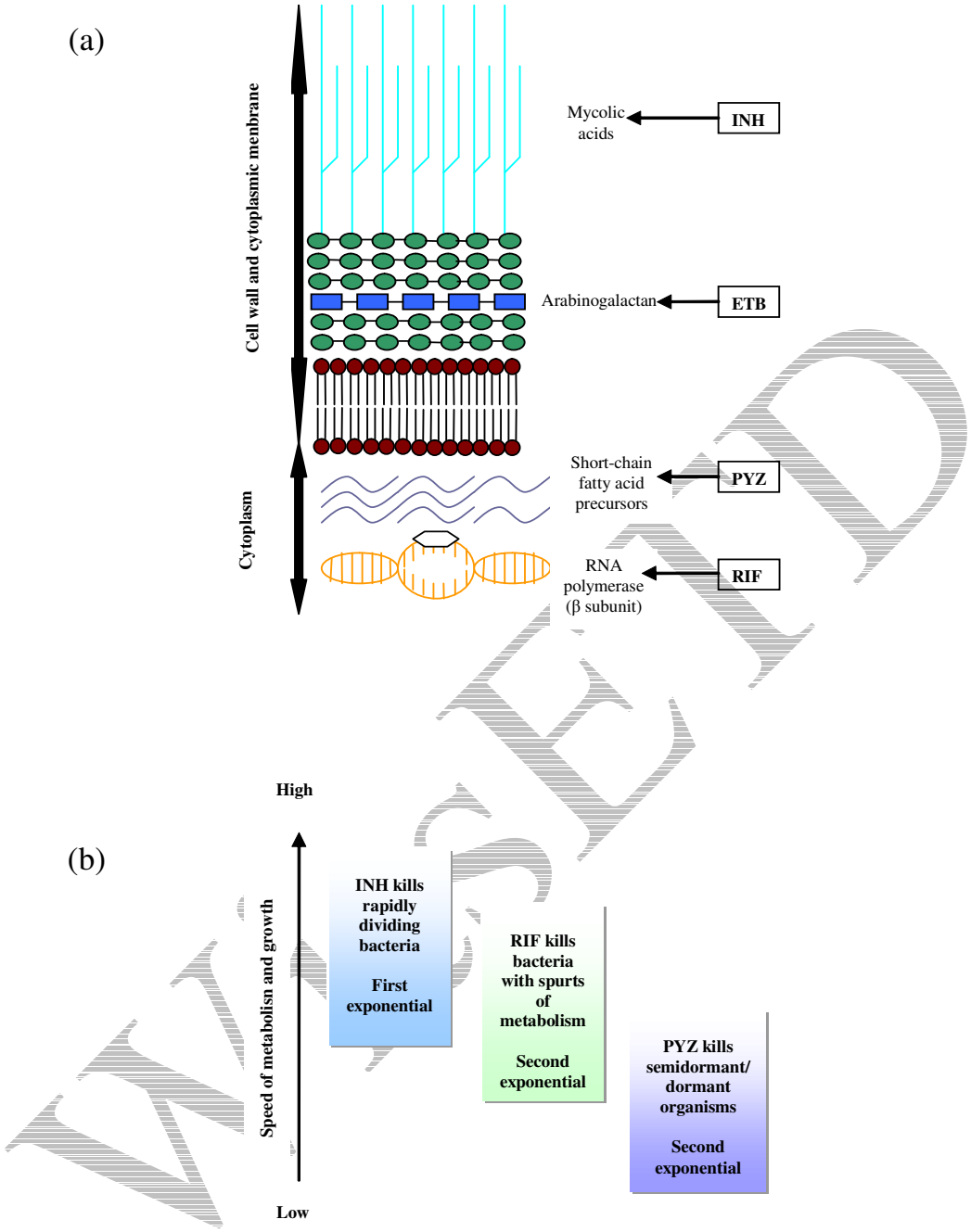

Figure 1.3: Action of anti-TB drugs: (a) sites of action of the principal anti-TB drugs (Parsons et al., Somoskovi et al., 2001; Rattan et al., 1998), (b) action of drugs according to their speed of growth at the start of treatment (Mitchison, 2005)

Table 1.2: Classes of anti-TB drugs

Agent	*Mechanism of Action*	*Activity Against M. tubercolosis*
Oral first-line agents		
Rifampicin (RIF)	Inhibits bacterial RNA synthesis by binding to the β subunit of bacterial DNA-dependent RNA-polymerase (DDRP) Inhibition of DDRP leads to blocking of the initiation chain formation in RNA synthesis. One of the most effective antituberculosis agents available and is bactericidal for intra- and extra-cellular bacteria. (Lund, 1994; Katzung, 2001).	RIF inhibits susceptible organisms at concentrations of less than 1µg/mL (Katzung, 2001).
Isoniazid (INH)	Most active drug for the treatment of TB caused by susceptibe strains. Is a pro-drug activated by katG, which exerts its lethal effect through inhibition of synthesis of mycolic acids, an essential component of mycobacterial cell walls, through formation of a covalent complex with an acyl carrier protein (AcpM) and KasA, a beta-ktoacyl carrier protein synthetase (Katzung, 2001; Williams and Lemke, 2002).	INH inhibits tubercle bacilli at a concentration of 0.2µg/mL (Katzung, 2001).
Pyrazinamide (PYZ)	Converted to the active pyrazanoic acid (encoded by pncA) by pyrazinamidase in susceptible organisms. Pyrazanoic acid lowers pH in the immediate surroundings of *M. tuberculosis* - organism unable to grow. May also function as an antimetabolite of nicotinamide and interfere with the synthesis of NAD, inhibiting the synthesis of short-chain, fatty-acid precursors (Katzung, 2001; Williams and Lemke, 2002).	Inhibits *M. tuberculosis* and other mycobacteria at concentrations of 20µg/mL (Katzung, 2001)
Ethambutol (ETB)	Inhibits mycobacterial arabinosyl transferases (encoded by the embCAB operon) involved in the polymerization of D-arabinofuranose to arabinoglycan, an essential cell wall component (Katzung, 2001; Williams and Lemke, 2002)	ETB is generally bacteriostatic, but at high doses (25mg/kg) can be bactericidal (Jindani et al., 1980). Inhibits susceptible strains of *M. tuberculosis* at concentrations of 1-5µg/mL (Katzung, 2001).
Injectable aminoglycosides Streptomycin, kanamycin, amikacin, capreomycin	The aminoglycosides are irreversible inhibitors of protein synthesis through binding to specific 30S-subunit ribosomal proteins (Katzung, 2001).	Bactericidal. *In vitro* and *in vivo* clinical data support use (Sanders et al., 1982; Heifets and Levy, 1989; Peloquin, 1993; Blaser et al., 1995).
Fluoroquinolones Ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin, sparfloxacin	Inhibit bacterial DNA synthesis through inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV, which are responsible for the relaxation of supercoiled DNA and the separation of replicated chromosomal DNA, respectively (Katzung, 2001).	Bactericidal, broad-spectrum antibacterials (Williams and Lemke, 2002). *In vitro* and *in vivo* clinical data support use (Kohno et al., 1992; Kennedy et al., 1996). Ciprofloxacin and levofloxacin inhibit strains of *M. tuberculosis* at concentrations of less than 2µg/ml. Newer agents (moxifloxacin, gatifloxacin, sparfloxacin) have lower minimum inhibitory concentrations (Rastogi and Goh, 1991; Ji et al., 1998; Katzung, 2001).
Bacteriostatic second-line drugs		
Ethionamide	Chemically related to INH, converted via oxidation to ethionamide sulfoxide, blocks the synthesis of mycolic acids Katzung, 2001; Williams and Lemke, 2002).	Inhibits most tubercle bacilli at concentrations of 2.5µg/mL or less (Katzung, 2001).
Cycloserine	Structural analogue of D-alanine, inhibits incorporation of D-alanine into peptidoglycan pentapeptide through inhibition of alanine racemase (Katzung, 2001).	Inhibits strains of *M. tuberculosis* at concentratins of 15-20 µg/mL (Katzung, 2001).
P-aminosalicylic acid	Anti-metabolite interfering with incorporation of para-aminobenzoic acid into folic acid - folate synthesis antagonist Katzung, 2001; Williams and Lemke, 2002).	Inhibits tubercle bacilli at concentrations of 1-5µg/mL (Katzung, 2001).
Other drugs	Potentially useful agents with conflicting animal or clinical evidence or agents with unclear efficacy because of possible cross-resistance	
Clofazimine	Unknown, but may involve DNA binding (Katzung, 2001). Possesses direct antimycobacterial and immunosuppressive properties (Williams and Lemke, 2002).	Bacteriostatic. MIC 90 <1·0mg *in vitro* (Bastian and Portaels, 2000). Concentrations attainable *in vivo*, particularly in macrophages (Jaganneth et al., 1995; Reddy et al., 1996).
Amoxicillin/clavulanic acid	Amoxicillin (a penicillin) inhibits cell wall synthesis. Clavulinic acid is a beta-lactamase inhibitor	β lactams in combination with beta lactamase inhibitors bactericidal in vitro (Cynamon and Palmer, 1983; Chambers et al., 1998; Donald et al., 2001).
Clarithromycin	Inhibition of protein synthesis via binding to 50S ribosomal RNA as aminoacyl translocation reactions and the formation of initiation complexes are blocked (Katzung, 2001).	Although *in vitro* antimycobacterial properties reported, data from animal and *in vivo* studies conflicting (Cavalieri et al., 1995; Hoffner et al., 1997; Luna-Herrara, 1997; Mor and Esfandiari, 1997).
Rifabutin	Activity is similar to that of rifampicin. Inhibits bacterial RNA synthesis by binding strongly to the β subunit of bacterial DNA-dependent RNA-polymerase (Katzung, 2001).	May be useful against some isolates of MDR TB (resistant to RIF *in vitro* but sensitive to rifabutin) (Mukherjee et al., 2004). Effective in prevention and treatment of disseminated atypical mycobacterial infection in AIDS patients with CD4 counts < 50 (Katzung, 2001).
Thiacetazone	Not clearly elucidated.	*In vivo* and *in vitro* evidence of bacteriostatic activity. Cross-resistance frequently seen between thiacetazone and both INH and ethionamide (Heifets, 1990; Okwera, 1994).

Table 1.3: Synopsis of novel anti-TB drug delivery systems

Drug	Delivery System and Polymer	ROA	Preparatory Methods	Characterisation Studies and System Suitability	Reference
INH	Porous, non-porous and hardened microparticles employing PLG	SC injection	Double emulsification solvent evaporation	Size: Mean volume diameters were: 62.11 µm, 71.95µm and 11.75 µm for porous, non-porous and hardened microparticles, respectively. *In vitro* studies: Sustained release of INH for up to 6 days from non-porous microparticles. Porous microparticles released INH over 3 days. Hardened PLG microparticles sustained release of INH for up to 7 weeks. *In vivo* disposition studies *(in mice)*: Porous and non-porous microparticles released INH in plasma for up to 2 days. Hardened PLG microparticles sustained release of INH for up to 7 weeks. Concentrations of INH obtained were higher than the MIC of INH.	Dutt and Khuller (2001a)
RIF, INH, PYZ, ETB	Microparticles employing PLG	Oral, singly or in combi-nation	Double emulsification solvent evaporation	DEE: 8–10% for PYZ; 10–11% for INH and 12–18% for RIF. Size: Diameters were 1.11µm for INH, 1.40µm for RIF and 2.20µm for PYZ microparticles. *In vitro* studies: Entrapped drugs were released in a sustained manner. In the intestinal fluid drug release was obtained up to 20 days *In vivo* studies: Entrapped drugs remained in circulation up to 72h as compared to free drugs (eliminated within 24h). Level of PLG encapsulated INH was found to be higher than its MIC value (0.1 µg/ml). Pharmacokinetic analysis *(PLG encapsulated drugs and free drugs)*: Increased C_{max}; AUC_{o-a}; $t_{1/2}$ (a) and $t_{1/2}$ (e) when drug were given entrapped in PLG microparticles indicated the potential of PLG for effective treatment of TB.	Ain et al. (2002)
RIF, INH, PYZ	Nanoparticles employing PLG	Oral	Multiple emulsion technique	Size: Majority (>80%) in the size range of 186–290nm, polydispersity index of 0.38±0.04. DEE: 56.9±2.7% for RIF, 66.3±5.8% for INH and 68±5.6% for PYZ. Drug loading: 570 to 680mg drug per gram of polymer. *In vitro* studies: drug release profile in PBS showed an initial (up to 48h) burst release followed by a negligible release of either drug extending up to 6 weeks. *In vivo* studies (*experimental infection and chemotherapy):* Following 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.	Pandey et al. (2003)
RIF, INH	Osmotically regulated capsular multi-drug oral delivery system employing HPMC and NaCMC	Oral	Phase inversion process – precipitation of membrane structure on a stainless steel mould pin	SEM: porous structure of the membranes. *In vitro* studies: sustained release of RIF and INH, with initial burst release, which may be sufficient to achieve MIC in blood. Thereafter, the system sustained the release of the drugs in a near-zero-order rate. *In vitro* release kinetics: first order kinetics. Statistical analysis of release rate data - modified asymmetric system the preferred system.	Prabakaran (2004)
RIF	Microspheres employing PLG	Inhaled/ aerosol	Solvent evaporation	Size: reported for 2 formulations - 3 to 4µm, and distribution demonstrated a Gaussian curve. *In vitro* studies: best *in vitro* release patterns, resulted in 21 and 12% cumulative *in vitro* drug release, respectively, after 6 days Release in monocytic cell lines *(murine J774 and the human Mono Mac 6)*: Bioassay assessment of cell culture supernatants from monocyte cell lines - release of RIF during a 7-day experimental period. Treatment of *M. tuberculosis* H37Rv-infected monocyte cell lines with RIF-loaded microspheres resulted in a significant decrease in numbers of CFU at 7 days following initial infection.	Barrow et al. (1998)
INH, RIF	Microparticles employing PLG	SC, Inhaled	Double emulsification solvent evaporation	Size: Volume mean diameters of 11.75µm (INH-loaded) and 11.64µm for (RIF-loaded) DEE: 10–11% (INH-loaded) and 12–14% (RIF-loaded) *In vivo*-combination drug disposition studies and experimental infection and chemotherapy studies: single dose of PLG microparticles - sustained release of INH and RIF for up to 7 and 6 weeks, respectively. Free drugs (in combination) injected in the same doses were detectable *in vivo* up to 24 h only. One dose of PLG microparticles cleared bacteria more effectively from lungs and liver in an experimental murine model of TB, as compared with a daily administration of the free drugs. Phase I trials underway.	Dutt and Khuller (2001b)

INH, RIF	Microspheres employing PLG	Inhaled	Microspheres: combination of solvent extraction and evaporation	Size: mean diameter of 6.214μm and only 38% of the microspheres fell in the size range of 0.5–3μm. *In vivo* studies: Microspheres were tested for uptake by murine macrophages in culture and resultant intracellular drug concentrations. The extent of microparticle delivery *in vivo* was examined by flow-cytometry. Drug concentrations (blood and alveolar macrophages) estimated after oral, vascular, intratracheal, and inhalation administration. Large numbers of particles delivered to the bronchiopulmonary system through a 2-minute exposure to fluidised particles. The intracellular drug concentrations resulting from vascular delivery of soluble drugs were lower than those resulting from particle inhalation.	Sharma et al. (2001)
RIF	Microparticles employing PLGA	Inhaled	Spray drying	Size: Volume median diameters (VMD) and geometric standard deviations (S.D.) were [VMD (μm)/geometric S.D.]: RIF-PLGA, 2.76/1.57; PLGA, 2.87/1.45; and RIF alone, 3.83/1.75. *In vivo* studies: Alveolar macrophage TB(H37Rv)-infected guinea pig model was used to screen for targeted delivery to the lungs by insufflation (with lactose excipient) or nebulization RIF-PLGA microspheres. Animals treated with single and double doses of RIF. PLGA microspheres – reduced numbers of viable bacteria, inflammation and lung damage compared with RIF-only treated animals 28 days post-infection. Two doses of RIF-PLGA – reduced splenic enlargement.	Suarez et al. (2001)
RIF	Microparticles employing PLGA	Inhaled	Solvent evaporation and spray drying	Morphology: spray dried rifampicin loaded PLGAmicroparticles were shriveled, unlike the spherical particles produced bysolvent evaporation. Size: Median diameters by volume were 3.45μm (solvent evaporation) and 2.76μm (spray drying). DEE: 20% (solvent evaporation) and 30% (spray dried). Particles are being evaluated in an animal model of TB.	O'Hara and Hickey (2000)
Ionizable prodrug of INH, isoniazid methane-sulfonate (INHMS)	Spherical microparticles employing PLA	Inhaled	Precipitation with a compressed antisolvent process	Drug loading efficiency: 93 to 152% Size: aerodynamic diameters ranged from1 to 3μm *In vitro* studies: Release profiles displayed two phases of drug release that were characterized by an initial burst effect, followed by a period of slower release *In vivo* studies *(drug accumulation in cultured rat alveolar macrophages)*: liquid chromatographic tandem mass spectrometric (LC-MS/MS) assay developed detected high level of INH in NR8383 (rat AM cell line) following exposure to drug-loaded microparticles. Compared the INH levels in lavaged bronchoalveolar macrophages by LC-MS/MS after Sprague–Dawley rats were administered either INHMS in PLA microparticles by intra-tracheal instillation or INH solution by gavage or intra-tracheal instillation - sustained delivery of INH to alveolar macrophages. Reduction in the blood levels of acetylisoniazid (AcINH), a major and potential toxic metabolite of INH.	Zhou et al. (2005)
RIF	Aerosolised liposomes formulated using Egg PC- and Chol-based liposomes	Inhaled	Neutral liposomes were prepared by cast film method	Modification: Imparted negative charge (DCP) or coated with alveolar macrophage-specific ligands (MBSA and *O*-SAP). Size: neutral and negatively charged liposomes composed of PC: Chol: DCP had average vesicle size of 2.32±0.48μm and 2.50±0.54μm, respectively. MBSA-coated liposomes size: 3.64±0.65μm, *O*-SAP-coated vesicles size: 3.85±0.59μm. DEE: 47.4±2.7%. *In vivo* studies: Percent viability of *Mycobacterium smegmatis* inside macrophages (*in vitro*) after administration of drug (*in vivo*) was 7–11% (ligand-anchored liposomal aerosols), 45.7 and 31.6% in case of plain drug and plain neutral liposomal aerosol (based on PC: Chol)-treated macrophages. Preferential accumulation of MBSA- and *O*-SAP-coated formulations in alveolar macrophages. Drug was estimated in the lung in high concentration even after 24h.	Vyas et al. (2004)
RIF, INH and PYZ	Nebulised SLNs prepared from nanocrystalline lipid suspensions in water	Inhaled	Emulsion solvent diffusion technique	Size: favourable mass median aerodynamic diameter suitable for bronchoalveolar drug delivery *In vivo* studies: Therapeutic experimental TB drug concentrations were maintained in the plasma for 5 days and in the organs for 7 days whereas free drugs were cleared by 1–2 days.	Pandey and Khuller (2005)

INH, RIF, PYZ and RIF, INH, PYZ, ETB	Nanoparticles employing alginate	Inhaled	Cation-induced gelification of alginate	Size: 235.5±0nm in size, with majority of particles (80.5%) in the respirable range, with mass median aerodynamic diameter of 1.1±0.4μm and geometric standard deviation of 1.71±0.1μm. DEE: 70–90% for INH and PYZ and 80–90% for RIF and 88–95% for ETB. *In vivo* studies *(disposition studies and chemotherapeutic studies)*: The formulation was orally administered to mice at two dose levels. A comparison was made in mice receiving free drugs at equivalent doses. Relative bioavailabilities of drugs encapsulated in alginate nanoparticles significantly higher compared with oral free drugs. Drug levels were maintained at or above the MIC 90 post nebulisation until Day 15 in organs (lungs, liver and spleen) after administration of encapsulated drugs, whilst free drugs stayed at or above the MIC 90 up to Day 1 only, irrespective of dose. Clinical trials are envisaged in the future.	Zahoor et al. (2005, 2006)
INH	Implant prepared from PLGA	Depot	PLGA polymer rods	*In vivo* studies: Rods implanted on the back of the rabbits under anaesthesia. Concentrations of INH and acetylisoniazid in serum and urine determined by HPLC. Concentrations of INH>0.2 μg/ml were found both in serum and urine up to 63 days after implant. Urine specimen obtained at 6 weeks after implantation demonstrated inhibition of growth of *M. tuberculosis in vitro* measured by the radiometric (Bactec) method.	Kailasam et al. (1994)
INH, PYZ	Single implants prepared from PLGA	Depot	Depot drug preparation	*In vivo* studies: 3 times the daily dose of PYZ contained in single PLGA polymer implant – no abnormally high (burst) levels of the drug evident after administration - sustained levels up to 54 days. Chemotherapeutic activity (investigated in mice) of the single PLGA polymer implants similar to standard oral treatment with the two drugs given daily for the entire 8 weeks, as judged by mortality and CFU counts of tubercle bacilli from lungs and spleen.	Gangadhara m, et al. (1999)

Key: CFU=colony-forming units, DEE= drug entrapment efficiency, SC=subcutaneous, HPMC=hydroxypropylmethylcellulose, NaCMC=sodium carboxymethylcellulose, MIC=minimum inhibitory concentration, PC=phosphatidylcholine, Chol=cholesterol, DCP= dicetylphosphate, MBSA=maleylated bovine serum albumin, and *O*-SAP=*O*-steroyl amylopectin, SLNs=solid lipid nanoparticles

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1.3. Novel Drug Delivery Systems for the Treatment of TB

Chemotherapy of TB is complicated by the requisite for multi-drug regimens that need to be administered over long periods. Poor patient compliance is the single most common reason for chemotherapy failure of TB (Prabakaran, 2004). To minimise toxicity and improve patient's compliance, extensive progressive efforts have been made to develop various implant-, nano- and microparticulate-, and various other carrier-based drug delivery systems that either target the site of *M. tuberculosis* infection or reduce the dosing frequency, which forms an important therapeutic strategy for the improvement of patient outcomes (Falk, 1997; Pandey et al., 2003). The systems under discussion employ either biodegradable polymers or systems requiring removal after use, and can release the drug either by membrane or matrix-controlled diffusion. Pertinent points are summarised in Table 1.3.

Recent trends in controlled drug delivery have seen microencapsulation of pharmaceutical substances in biodegradable polymers as an emerging technology. Carrier or delivery systems such as liposomes and microspheres have been developed for the sustained delivery of anti-TB drugs and have demonstrated better chemotherapeutic efficacy when investigated in animal models (e.g. mice) (Falk, 1997). Anti-TB drugs have been successfully entrapped and delivered in biodegradable polymers such as poly (DL-lactide-co-glycolide) (PLG) (Kailasam et al., 1994), which are biocompatible and release drug in a controlled manner at therapeutic levels. Dutt and Khuller (2001a) entrapped INH and RIF, in PLG polymers for injection as a single dose. This provided sustained release of drugs over 6–7 weeks when tested in mice. Dutt and Khuller (2001a, 2001b) have entrapped INH and RIF in PLG polymers. When injected subcutaneously as a single dose, the microparticles, having a diameter ranging from 11.75µm to 71.95µm, provided sustained release of drugs over 6–7 weeks when tested in mice (Dutt and Khuller, 2001a). The authors previously observed that particles with a size range >10µm remained at the site of

injection forming a depot. The entrapped contents of the microparticles were gradually released by diffusion through the polymeric particles. Such depots can yield release profiles extending over several months until the entire polymeric device is biodegraded.

However, these formulations have to be injected either subcutaneously or intravenously, and the pain and discomfort associated with these routes of administration, in general, is not acceptable. Hence, there is a continuous need to develop an oral drug delivery system that is convenient for patients (Prabakaran, 2004).

Amidst these concerns, Ain et al. (2002) reported the pharmacokinetics of PLG encapsulated anti-TB drugs; orally administered either individually or in combination in mice. A study conducted by Pandey et al. (2003) reported the formulation of three frontline anti-TB drugs, i.e. RIF, INH and PYZ encapsulated in PLG nanoparticles. 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. Therefore, oral nanoparticle-based anti-TB drug therapy can allow for a reduction in dosing frequency for enhanced management of TB. Prabakaran (2004) developed an osmotically regulated capsular multi-drug oral delivery system comprising asymmetric membrane coating- and dense semipermeable membrane coating-capsular systems for the simultaneous controlled administration of RIF and INH for the treatment of TB (Figure 1.4(a)). This was in an attempt to reduce the problems associated with multi-drug therapy. The modified asymmetric system provided satisfactory sustained release of RIF and INH, with initial burst release, which may be sufficient to achieve minimum effective concentration in blood. Thereafter, the system sustained the release of the drugs in a near zero order rate - an ideal release profile for controlled drug delivery. In turn; this system has the potential to improve the safety profile of the drugs and enhance the activity duration of drugs

exhibiting short half-lives. The once daily system is optimal, and could potentially enhance patient compliance.

In addition to these combinations, over the past several years a number of different types of RIF-only controlled release formulations have been developed to improve clinical efficacy of the drug as well as patient compliance (Mathur et al., 1985; Uppadhyay et al., 1997; Schierholz, 1997; Deol and Khuller, 1997; Denkbas et al., 1995; Amar et al., 1997; Nakhare et al., 1995; Khopade et al., 1996; Barik et al., 1993; Sreenivasa Rao and Murthy, 2002).

Further attempts to solve the difficulties inherent in multi-drug therapy have included the development of microparticulate preparations to target alveolar macrophages that harbour *M. tuberculosis* (Barrow et al., 1998; Anisimova et al., 2000; Dutt and Khuller, 2001b; Sharma et al., 2001; Ahsan et al., 2002; Makino et al., 2004). In the case of pulmonary TB, delivering the drug directly to the site of infection through inhalation of an aerosolised delivery system has the inherent advantages of bypassing first-pass metabolism and maintaining local therapeutically effective concentrations with decreased systemic side effects (Zhou, 2005). Because *M. tuberculosis* is known to infect alveolar macrophages, which have a fundamental effect on the pathogenic progression of TB, there have been renewed interests in targeting of anti-TB drugs to these cells. One of the most extensively studied carriers for targeting alveolar macrophages employs biodegradable polymeric micro- or nanoparticulate systems (Barrow et al., 1998; Anisimova et al., 2000; Dutt and Khuller, 2001b; Sharma et al., 2001; Ahsan et al. 2002; Pandey et al., 2003; Makino et al., 2004).

Despite the success of these systems in targeting and providing sustained release of anti-TB drugs to alveolar macrophages, a number of the methods used to generate particles in these studies vary

in their capability for the production of reproducible particles with the optimum size for inhalation therapy (i.e. <5μm). Barrow et al. (1998) formulated RIF-loaded microspheres using the method of solvent evaporation, aiming to maintain a size of 1 to 10μm. Only the size distributions of two formulations were reported, being 3 to 4μm, and the distribution assumed a Gaussian curve. Dutt and Khuller (2001b) encapsulated INH and RIF into hardened PLG microparticles by a double emulsification solvent evaporation procedure, and these had a resultant volume mean diameter of 11.75μm for INH microparticles and 11.64 μm for RIF microparticles. These are currently undergoing Phase I trials. Sharma et al. (2001) incorporated both INH and RIF into PLG microspheres using a combination of solvent extraction and evaporation, but again these particles had a mean diameter of 6.214μm and only 38% of the microspheres fell in the size range of 0.5–3μm. Suarez et al. (2001) attained the targeted delivery of RIF microparticles to the lungs. O'Hara and Hickey (2000) succeeded in obtaining RIF-loaded PLG particles with median diameters by volume of 2.76μm and 3.45μm by spray drying and solvent evaporation respectively. Zhou et al. (2005) did achieve the formulation of spherical microparticles between 1 and 3μm in diameter (Figure 1.4(b)). The microparticles, prepared by the precipitation with a compressed antisolvent process, were evaluated for their potential in targeting an ionisable prodrug of INH, isoniazid methanesulfonate (INHMS), for sustained delivery of INH to alveolar macrophages. South African researchers are investigating the synthesis of polymeric nanoparticles incorporating anti-TB agents employing the biodegradable polymers PLG, alginate-chitosan and poly(caprolactone). Drug-free nanoparticles have thus far been synthesised, formulated using both double emulsion solvent evaporation and spray drier techniques, having a size ranging from 150-500 nm (Figure 1.4(c)) (CSIR, 2005).

Most recently Zahoor et al. (2005, 2006) undertook pharmacokinetic and chemotherapeutic studies with aerosolised alginate nanoparticles encapsulating INH, RIF and PYZ and RIF, INH,

PYZ and ETB. The nanoparticles were prepared by cation-induced gelification of alginate and were 235.5±0nm in size, with drug encapsulation efficiencies of 70–90% for INH and PYZ and 80–90% for RIF and 88–95% for EMB. The majority of particles (80.5%) were in the respirable range, with mass median aerodynamic diameter of 1.1±0.4μm and geometric standard deviation of 1.71±0.1μm. The chemotherapeutic efficacy of three doses of drug-loaded alginate nanoparticles nebulised 15 days apart was comparable with 45 daily doses of oral free drugs. Thus, inhalable alginate nanoparticles can serve as an ideal carrier for the controlled release of anti-TB drugs. Clinical trials are envisaged in the future for evaluation of this system before use in humans.

Pandey and Khuller (2005) recently evaluated the chemotherapeutic potential of nebulised solid lipid nanoparticles (SLNs) incorporating RIF, INH and PYZ against experimental TB. SLNs are nanocrystalline suspensions in water, prepared from lipids, which are solid at room temperature. The SLNs, prepared by the emulsion solvent diffusion technique, possessed a favourable mass median aerodynamic diameter suitable for bronchoalveolar drug delivery. Following a single nebulisation to guinea pigs, therapeutic drug concentrations were maintained in the plasma for 5 days and in the organs for 7 days whereas free drugs were cleared after 1–2 days.

From these works, it is apparent that the general thinking is to synthesise particles with mean diameters of less than 5μm. Investigation by Edwards et al. (1997), however, demonstrated an alternative slant to efficacy in pulmonary drug delivery: particles with mass densities $<0.4 g/cm^3$ and mean diameters exceeding 5μm were inspired deep into the lungs and escaped the lungs' natural clearance mechanisms until the inhaled particles delivered their therapeutic payload.

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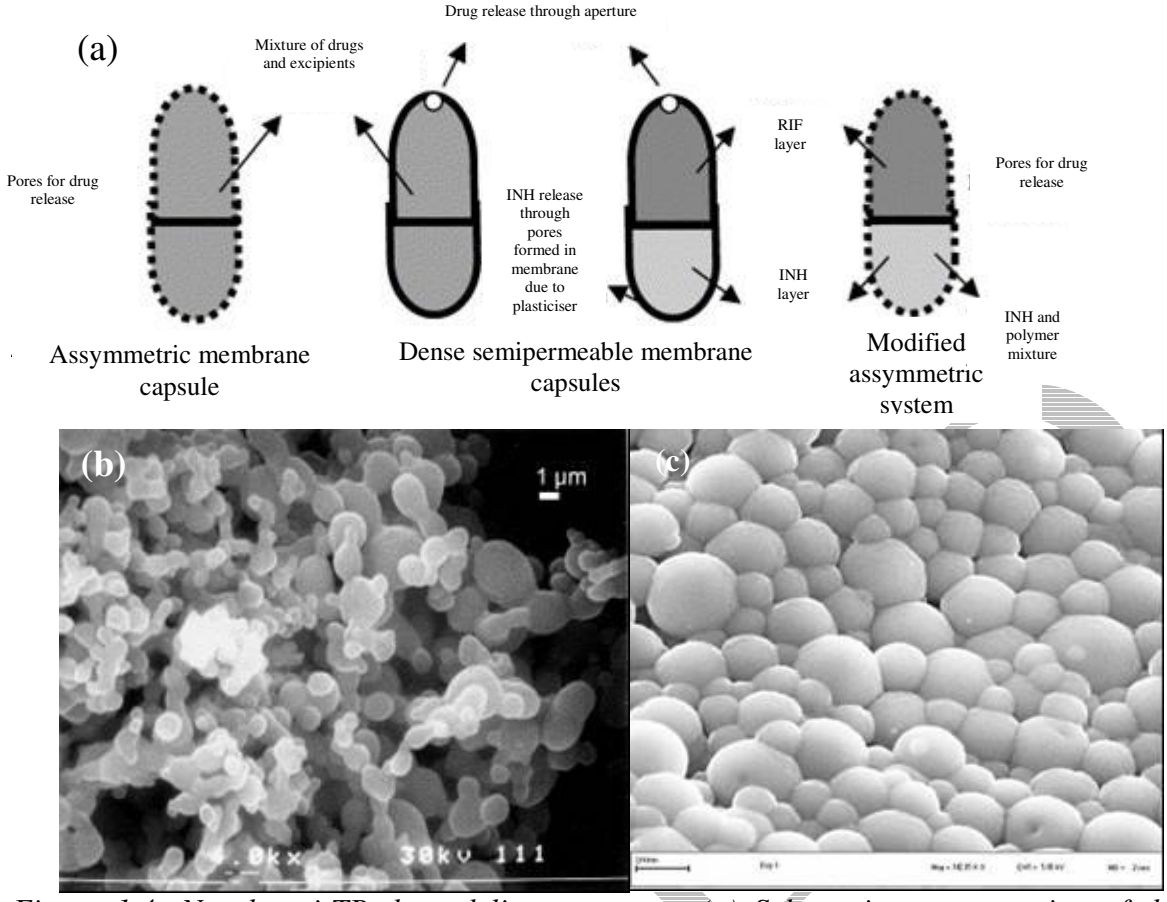

Figure 1.4: Novel anti-TB drug delivery systems: (a) Schematic representation of design of various capsular systems for simultaneous controlled delivery of INH and RIF (Prabakaran, 2004) (b) Representative SEM of anti-TB drug-loaded PLA microparticles produced by compressed antisolvent processing for targeted and sustained delivery of INH to alveolar macrophages (c) Representative SEM of anti-TB drug-loaded PLGA nanoparticles (CSIR, 2005).

In another approach to solve the predicament of poor patient compliance, depot-delivery of anti-TB drugs has been investigated. Studies have demonstrated that a single implant of INH in polylactic-co-glycolic acid (PLGA) copolymer could ensure sustained levels of free INH for a period of up to 8 weeks following implantation in rabbits (Kailasam et al., 1994). Gangadharam et al. (1999) also investigated the chemotherapy of TB in mice using single implants of INH and PYZ.

A number of the aforementioned developments in modified drug delivery are encouraging and represent attractive options with significant merit and no attempt is made to improve on these

exemplary studies, however, many suffer the disadvantages of higher cost, use of toxic substances during preparation, immobilisation at the site of implantation and surgical requirements (Zahoor, 2005). Furthermore, the need to develop an oral drug delivery system with improved patient acceptance is affirmed by the accelerated pace of oral drug delivery system development fostered by the need to deliver medications to patients more efficiently and with fewer side effects, especially in developing countries.

1.4. Statement of Problem

The WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) encourage use of Fixed Dose Combination (FDC) formulations – this evolved from the fact that TB always requires multi-drug treatment (Blomberg and Fourie, 2003). Patients should thus be given FDCs adjusted for body weight whenever self-administration of anti-TB drugs is permitted (WHO, 1999). A FDC - which is a combination of two or more first-line anti-TB drugs in a single formulation at a fixed proportion - prevents monotherapy; and it is expected that this will reduce the emergence of drug resistant TB; simplify treatment, and thus minimise prescription error and increase patient and doctor compliance; simplify drug stock management, shipping and distribution; and reduce the risk of misuse of RIF for conditions other than TB (WHO, 1999).

Of dire concern, however, is the issue of unacceptable RIF bioavailability in a number of FDC anti-TB formulations (Panchagnula and Agrawal, 2004). This variable bioavailability of RIF is considered as a major obstacle in the effective implementation of FDCs in national TB programs and thus successful treatment of the same (Blomberg et al., 2001; Blomberg and Fourie, 2003). The decomposition of RIF has varied from 8.5 to 50% in the acidic environment of the stomach in the time range corresponding to the gastric residence time for most dosage forms in humans (≈15 minutes to 105±45 minutes) (Coupe et al., 1991; Shishoo et al., 2001). However, the gastric-

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emptying time for some single-unit dosage forms may reach 6 hours (Digenis, 1994). The use of a substandard FDC will ultimately result in drug resistant TB and treatment failure (Pillai et al., 1999). The factors proposed for this variation in the bioavailability of RIF from different FDC formulations include the particle size and crystalline form of the drug, manufacturing process and the excipients employed (Laing et al., 1999; Blomberg et al., 2001). The effect of these factors, however, has not been convincingly explained in previous studies. RIF is known to undergo hydrolysis in acidic medium to the insoluble 3-formyl rifamycin SV (3-FRSV). INH accelerates degradation of RIF into this poorly absorbed derivative (3-FRSV) in the acidic environment of the stomach via reversible formation of the isonicotinyl hydrazone of 3-FRSV with INH (Singh et al. 2000a; Singh et al. 2000b; Shishoo et al., 2001; Mohan et al., 2003; Mariappan et al., 2004). Shishoo et al. (2001) has indicated that RIF in the presence of INH as a FDC may undergo greater decomposition in the gastric environment, as compared to when RIF is administered (orally) alone. Thus, less RIF will be available for absorption from FDCs as compared to RIF administered as a separate formulation. This will be reflected in the poor bioavailability from the former formulation.

There is thus an urgent need to modify the FDC formulation in such a way that RIF and INH are not released simultaneously in the stomach. Alternatively both drugs need to be administered separately after an interval corresponding to average gastric residence time, which is somewhat unpredictable due to high intra- and inter-subject variability (Coupe et al., 1991; Shishoo et al., 2001; Sankar et al., 2003).

Furthermore, this bioavailability reduction has been considerably reported when RIF is combined with other anti-TB agents in single oral solid formulations, and the unstable RIF undergoes rapid degradation in the presence of INH in the formulations forming the isonicotinyl hydrazone of 3-

FRSV. Mariappan et al. (2003) described the generation of this degradation product when suspensions of INH and RIF and FDC tablets were stored under accelerated stability conditions. It follows that physical isolation of RIF and INH within the FDC delivery system will improve drug stability during storage.

Fairly recently, Chen (2000) proposed a mechanism for this apparent degradation of RIF. 3-FRSV and INH could possibly undergo Schiff's reaction to form a complex (Figure 1.5(a)). The carbonyl groups and amine groups may rearrange to yield an iminium ion. The C-4 hydroxy group enhances the complex formation by possibly forming a hydrogen bond with the hydrogen atom attached to the nitrogen. This is a basic requirement of the Schiff's reaction. In addition, carboxylic acids and alcohols can also undergo carbonyl condensation reactions (McMurry, 1992). INH could react with RIF in this manner, which could account for the instability of RIF when present together with INH (Figure 1.5(b)). This interaction could also occur between RIF and PYZ, however, it has frequently been observed that INH caused further RIF stability reduction compared to PYZ. The reason for this is attributed to the fact that the carboxylic acids and alcohol further undergo Fischer's esterification (McMurry, 1992). The hydroxyl groups of RIF are readily able to react with the aqueous carboxylic acid degradants yielded by INH and PYZ to form an ester (Figure 1.5(c)). However, as PYZ lacks the electron-withdrawing group such as the secondary nitrogen found on the hydrazide group of INH, there are fewer tendencies for this reaction to be expected between PYZ and RIF.

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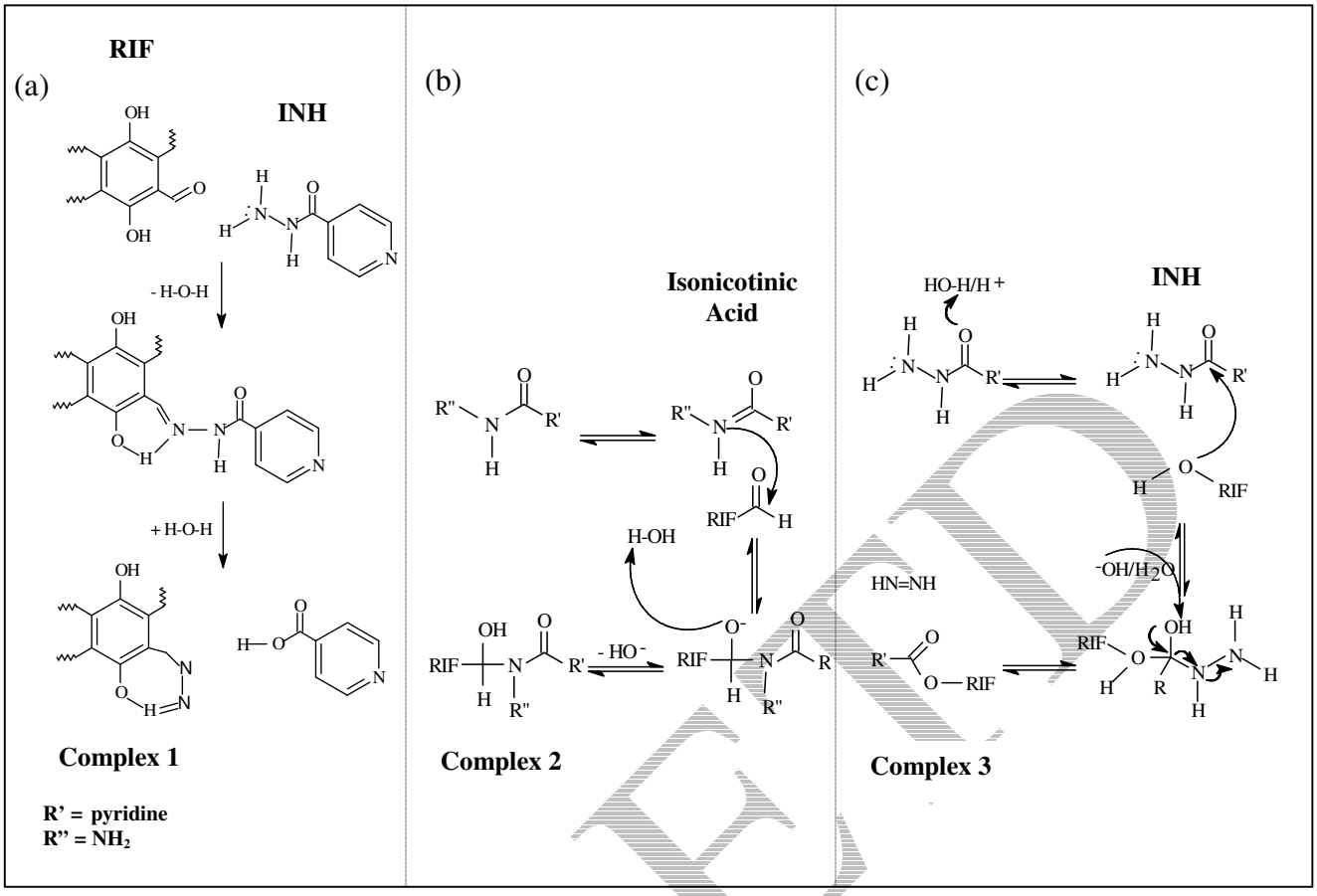

Figure 1.5: Proposed mechanisms for interaction between RIF and INH: (a) Schiff's Reaction of RIF and INH, (b) Carbonyl Condensation of RIF and INH, (c) Fischer's Esterification Reaction between RIF and INH (McMurry, 1992; Chen, 2000)

Permeability studies have demonstrated that RIF is well absorbed from the stomach due to its solubility, which has been shown to be maximal between pH 1-2. INH, although demonstrating solubility in the gastric environment, is comparatively well absorbed from all three segments of the small intestine. RIF and INH thus exhibit regional specific permeability, and the bioavailability problems associated with RIF could be overcome by developing an FDC in which the delivery of the two drugs is segregated, with RIF released in the stomach and INH in the small intestine, thus targeting their respective absorption windows (Mariappan and Singh, 2003). A FDC multiparticulate oral system, which boasts ease of manufacture and directly attacks RIF bioavailability concerns and poor patient compliance with existing with FDC anti-TB formulations, is yet to be globally developed.

1.5. Approach to the Problem -The rationale for the development of a novel polymer-based oral multiparticulate fixed dose combination anti-TB drug delivery system to achieve segregated delivery of RIF and INH

Drug delivery, which takes into consideration the carrier, the route and the target, has evolved into a strategy of processes or devices designed to enhance the efficacy of therapeutic agents through modified or controlled release. This may involve enhanced bioavailability, improved therapeutic index, or improved patient acceptance or compliance. Drug delivery has been defined by Flynn (1982) as 'the use of whatever means possible, be it chemical, physicochemical or mechanical, to regulate a drug's access rate to the body's central compartment, or in some cases, directly to the involved tissues'. The underlying principle that drug delivery technology can bring both therapeutic and commercial value to health care products has been widely accepted. This has created an intense need for presenting 'old' drugs, such as those encompassed in the anti-TB regimen, in new forms utilising novel forms of delivery and dosage forms (Ranade and Hollinger, 2003). Drug delivery is the key in realising the full therapeutic potential of these drugs.

The need for research into an oral anti-TB drug delivery system is thus warranted as the efficacy of the current regimen may be improved if the delivery rate, biodegradation, and site-specific targeting can be predicted, monitored, and controlled. From both a financial and a global health care perspective, finding new ways to administer the anti-TB drugs in oral form and delivering the multiple-dose, long-term therapies in inexpensive, potent, forms with improved bioavailability is needed. The provision of administration methods, embodied by a dosage form that addresses FDC bioavailability concerns, that will allow patients to safely treat themselves and enhance their compliance with the anti-TB regimen is a significant health care development, particularly in developing countries where access to doctors, clean syringes, sterile needles, and sophisticated treatments are few and far between.

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As mentioned, the major route of drug administration is through the oral cavity. This route provides the greatest comfort and convenience of dosing. In addition to avoiding the patient discomfort associated with the parenteral route, the accidental overdosing of the drug can be corrected by withdrawing the unabsorbed drug from the stomach. An anti-TB dosage form that can be orally administerd once daily would be optimal for patient compliance (Prabakaran, 2004).

In addressing oral bioavailabiliity concerns, chemical modification or pro-drug formation may well be successfully implemented to alter the pharmacokinetics of RIF and INH. Prodrug strategies have successfully improved the oral bioavailability of numerous compounds. In many cases, this involves masking a polar group by esterification to increase lipophilicity and enhance the extent of absorption from the gastrointestinal tract. After absorption, the ester is enzymatically hydrolysed to release the parent drug.

INH was synthesised in 1912 from ethyl isonicotinate and hydrazine by Meyer and Malley as part of their doctoral work in Prague. In 1945, its anti-TB properties were elucidated when nicotinamide was discovered to have anti-TB effects. This prompted the testing of other pyridine derivatives for their antimycobacterial effects. Being a pro-drug itself, activated through endogenous mycobacterial catalysis, various additional chemical miodifications have been invesigated to alter INH pharmacokinetics. Gianolla et al. (1992) attached various acyl groups to the amine ($-NH_2$) function of INH for improved lipophilicity and afforded good yields in pro-drugs, which were characterised by spectroscopic and analytical methods. Crooks et al. (2004) proposed the fabrication of and INH prodrug through the formation of covalent conjugates of INH with mono- di- and poly-oxaalkanoic or thiaalkanoic acids. The conjugation is purported to provide covalent compounds having a chemotherapeutic effect, with enhanced permeation of biological membranes, which remain intact until enzymatically cleaved. As reported, Zhou et al.

(2005) developed an ionisable prodrug of INH, INHMS, for sustained delivery of INH to alveolar macrophages. The charged prodrug was ion-paired with two different hydrophobic cations: tetrapentylammonium-and tetraheptylammonium-bromide. The prodrug required loading into microparticles for realisation of the targeted sustained effect.

RIF was developed in the Dow-Lepetit Research Laboratories (Milan, Italy) as part of an extensive program of chemical modification of the rifamycins, the natural metabolites of Nocardia mediterranei. Systematic structural modifications of most of the functional groups of the rifamycin molecule were performed with the objective of finding a derivative that was active when administered orally. The understanding of structure-activity relations in the rifamycins led to the synthesis of several hydrazones of 3-formylrifamycin SV. Among them, the hydrazone with N-amino-N'-methylpiperazine, RIF, was the most active in the oral treatment of infections in animals and, after successful clinical trials, was introduced into therapeutic use in 1968 (Sensi, 1983). To date, no form of RIF has been widely clinically applied that significantly improves on its oral bioavailability. There is little solubility advantage associated with polymorphic forms, which is inconsequential from a clinical and regulatory point of view (Agrawal et al., 2004).

A piperine composition for the improvement of gastrointestinal absorption and systemic utilisation of nutrients and nutritional supplements comprising an extract from the fruit of *Piper* containing a minimum of 98% of pure alkaloid piperine, has been added to multi-drug formulations for the treatment of TB and leprosy. A formulation, containing RIF, INH and PYZ and the said composition, has been tested in human volunteers (Indian Patent No. 1232/DEL/89). In the majority of cases, the comparative levels and peak concentration of the drugs in the presence of piperine were higher. The applicability of these results to bioavailability

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enhancement, which aims to lower dosage levels and shorten the treatment course, is apparent, but presently cost prohibitive in developing countries (Majeed et al., 1996).

Pro-drug formation is clinically relevant in altering *in vivo* disposition kinetics and in attaining sustained release, but developments have not necessarily addressed the deleterious RIF-INH interaction upon oral administration. Thus, in order to manufacture an oral system as cheaply and efficiently as possible, intrinsic drug delivery principles can be productively implemented, employing readily available polymeric and other formulatory excipients to segregate the delivery of the pure drugs. Shishoo et al. (2001) have promoted the need to develop a stable formulation containing the RIF-INH combination for differentiated gastrointestinal release and suggested formulation as enteric-coated tablets or alternative multilayered dosage forms.

In developing an oral modified-release system for anti-TB drugs, cognisance was taken of the increase in popularity of multiparticulate (or multi-unit) solid dosage forms (e.g. beads, pellets, granules, and micro- and nanoparticles) in the area of oral controlled drug delivery (Lippold, 1990). Formulation of an anti-TB dosage form as an oral multiparticulate drug delivery system would furnish many biopharmaceutical advantages when compared with solid single-unit dosage forms in terms of a more even and predictable distribution and transportation in the gastrointestinal tract that is fairly independent of the nutritional state, predictable gastrointestinal transit time, less localised gastrointestinal disturbances and greater product safety; as well as having an application in the improvement of patient compliance, as further expounded on in Chapter 2. In view of the many benefits offered by multiple-unit dosage forms, it is speculated that such systems are particularly useful for site-specific targeting within the gastrointestinal tract (Pillay and Fassihi, 1999; Schmidt and Bodmeier, 2001).

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The fabrication of a polymeric once-daily oral multiparticulate FDC of the principal anti-TB drugs, which attains segregated delivery of RIF and INH for improved RIF bioavailability, could be a step in the right direction in addressing issues of treatment failure due to patient non-compliance, the consequences of which are devastating.

1.6. Aims and Objectives of the Study: Description of the Oral Multiparticulate Drug Delivery System

The aim of the present study is to develop an oral pharmaceutical RIF-INH composition intended for facilitated oral administration that ensures differentiated release of RIF and INH in the gastrointestinal tract as conceptually illustrated in Figure 1.6(a). Current commercially available two-drug FDCs (300mg RIF and 150mg INH) for adult TB chemotherapy in South Africa comprise immediate-release film-coated tablets (average dimensions: 19mm x 8mm). In order to attain the fabrication of a once-daily FDC, the intended delivery system will be presented as a dry dispersible multiparticulate system incorporating enterosoluble and reconstitutable multiparticulate entities, their respective functionalities embodying the requirements of small intestinal INH delivery and immediate gastric availability of RIF. The dry dispersible multiparticulate system may be dispensed as single dose sachets for immediate reconstitution prior to administration to the patient for once-daily dosing as a compliance-promoting tool (Figure 1.6(b)). The suspension system is thus rationalised by the requisite to deliver RIF in a highly available form that will be readily absorbed from the gastric environment. Agrawal et al. (2004) have indicated that RIF release in the acidic medium is critical for its bioavailability.

INH, the hydrazide of isonicotinic acid, is a small (M_w=137g/mol), freely water-soluble molecule, thus targeting its release to the small intestine will be a delivery challenge in lieu of the shortcomings of the current enteric-release technologies. The INH will be entrapped within an

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enteric-release polymer, which may include methacrylic acid and ethyl acrylate copolymers, cellulose acetate phthalate, polyvinyl acetate phthalate, and hydroxypropyl methylcellulose acetate succinate for the formulation of enterosoluble multiparticulates. The preferred embodiment of the multiparticulates is a final size of less than 5000 microns and reproducible sphericity for ease of swallowing and delivery as dispersible multiparticulates.

The administration of a 300mg oral dose of INH results in a peak plasma concentration of 3-5µg/mL within 1 to 2 hours. Because the metabolism of INH, especially acetylation, by N-acetyltransferase is genetically determined, is must be considered here. The average concentration in the plasma for rapid acetylators is approximately one third to half of that in slow acetylators and average half-lives are less than 1 hour to 3 hours, respectively. The enhanced susceptibility of rapid acetylators to hepatotoxicity has been suggested but has yet to be proved. More rapid clearance of INH by rapid acetylators is of no therapeutic consequence when appropriate doses are administered daily, but subtherapeutic concentrations can occur if the drug is administered as a once-weekly dose (Katzung, 2001). The once-daily dose is thus rationalised. A more gradual delivery of INH to the designated absorption site from a modified delivery system may further reduce untoward effects, including the combinatory RIF and INH hepatotoxicity.

The dry suspension is provided as reconstitutable granules incorporating RIF for extemporaneous dispensing which may be freshly reconstituted prior to administration to the patient by adding water; and is prepared by mixing the RIF, in the form of a powder, and the suspension and granulation adjuvants. It may be granulated according to the wet granulation technique.

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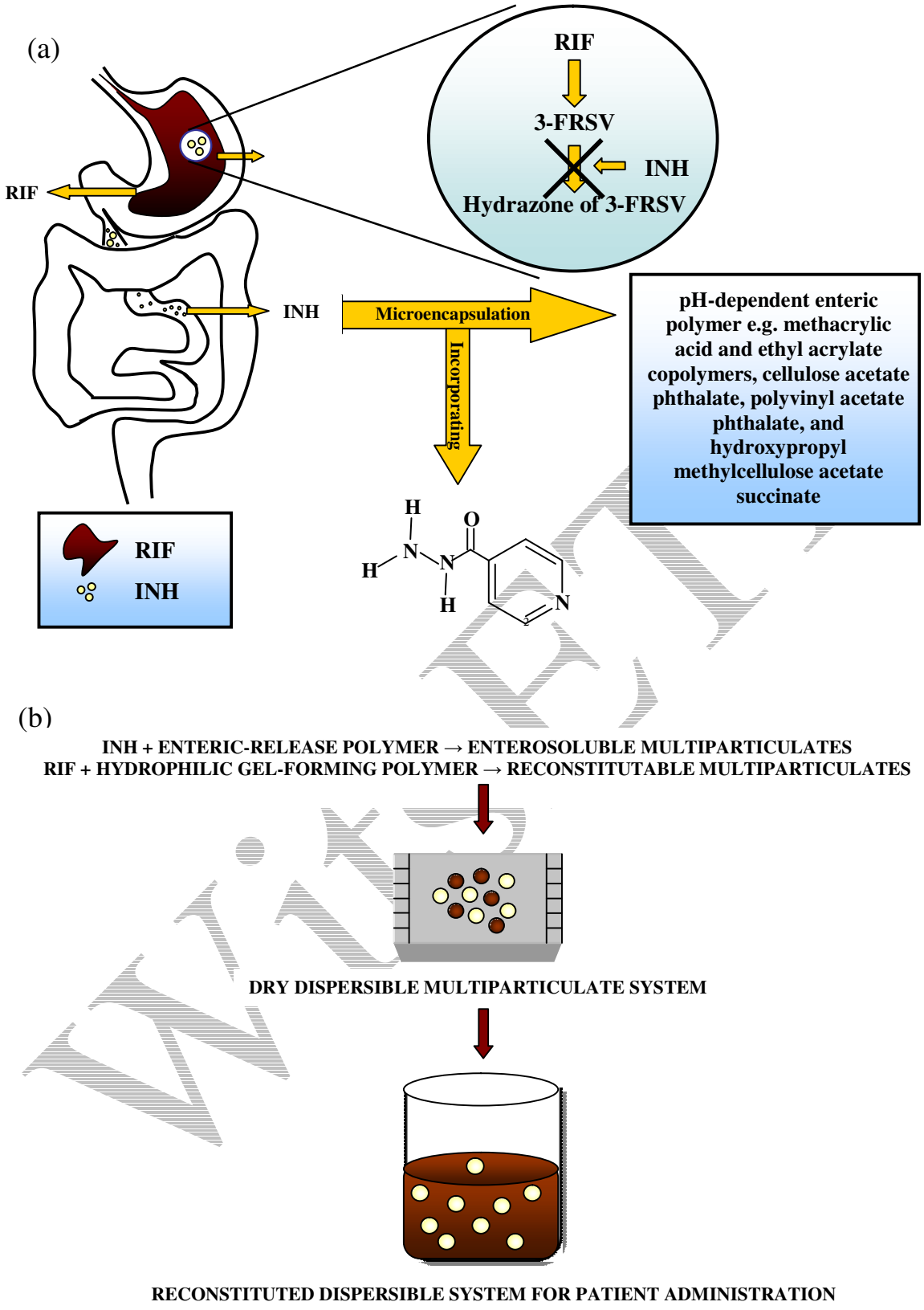

Figure 1.6: Diagrammatic representation of (a) the formulation strategy to attain differentiated gastrointestinal delivery in a single dose and (b) the final anti-TB drug delivery system

The physical properties of the drug to be incorporated in the granules for suspension must also be considered. RIF, a semi-synthetic rifamycin, is a relatively large molecule (M_w=822.95g/mol) that is only very slightly soluble in water (1g in approximately 762mL water). As a borderline class II drug of the Biopharmaceutic Classification System (BCS), being zwitter-ionic in nature, RIF shows pH-dependent solubility, with dissolution as the rate-limiting step affecting its absorption from gastrointestinal tract (Agrawal and Panchagnula, 2004). It is provided in the form of an odourless orange to red powder. A $1\%^{w}/_{v}$ suspension in water has a pH of 4.5 to 6.5 (Budavari, 1989). Whereas INH is stable for several weeks in buffered aqueous solutions below pH 8, RIF decomposes rapidly in acidic and alkaline conditions, but slowly at neutral pHs, with 3-FRSV being the main decomposition product of RIF in aqueous acidic medium (Florey, 1976; Florey, 1977). These considerations must be borne in mind during ensuing formulation processes and *in vitro* analyses, and further emphasises the requisite for delivery of RIF in a system that facilitates its immediate dissolution upon arrival in the stomach to limit acid degradation.

The oral pharmaceutical granules for suspension are required to contain at least one gel-forming viscosity-enhancing agent to ensure adequate suspension of the INH-loaded enterosoluble multiparticulates and the poorly water-soluble RIF. The appropriate agent/s is preferably selected from pharmaceutically acceptable viscosity agents, for example xanthan gum, hydroxypropylmethyl cellulose, methylcellulose, carageenan, carboxymethyl cellulose, microcrystalline cellulose, polyvinylpyrrolidone, soluble starches and carbomers. On disintegration of the granules, the agent/s must disperse rapidly to form a three-dimensional supporting network possessing the necessary attributes for extemporaneous use.

To achieve the aforementioned aims, the following objectives are highlighted:

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1. To review the diverse novel systems developed for the improved delivery of anti-TB drugs to the patient for promotion of enhanced therapetic outcomes and compliance and to rationalise the need for a novel oral system for segregated gastrointestinal delivery of RIF and INH.
2. To review the gastrointestinal physiology and enteric-polymer selection criteria for fabrication of an enterosoluble drug delivery system incorporating INH.
3. To identify a single feasible method for the formulation of an enterosoluble multiparticulate drug delivery system incorporating INH.
4. To experimentally synthesise several variants of the preferred enterosoluble multiparticulate system employing a Box-Behnken Response Surface Design to elucidate the effect of independent variables; the upper and lower limits of which were set during preliminary investigations. This will facilitate a mechanistic evaluation of possible correlations between independent variables.
5. To determine the optimum parameters to synthesise an ideal polymeric enterosoluble multiparticulate system based on statistical optimisation implemented via the Response Surface Methodology.
6. To elucidate the physicochemical dynamics and characteristics of the optimised enterosoluble multiparticulate system through determination of its thermal transitions, vibrational transitions, crystalline structure, thermal diffusivity, degree of crosslinking and erosional dynamics under simulated gastrointestinal conditions.
7. To identify a suitable suspending and gel-forming polymer or combination of agents that will rapidly form a suspension in tepid water.
8. To elucidate the optimum combination and level of suspending and gel-forming polymers employing a Face-Centred Central Composite Design for incorporation within RIF-loaded reconstitutable multiparticulates, having acceptable flow properties.

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9. To assess the *in vitro* dissolution characteristics of the optimum RIF-INH multiparticulate combination to ascertain segregated delivery. The proposed United States Pharmacopoeial (USP) high performance liquid chromatographic (HPLC) and colorimetric method, and regressional analysis of ultraviolet (UV) spectrophotometric absorbance data, may be employed to resolve RIF and INH.
10. To investigate current trends in nanotechnology for the preliminary design of a nanoparticulate anti-TB delivery system.

1.7. Overview of the Dissertation

The dissertation was deconstructed as follows for attainment of the aforementioned aims, with Figure 1.7 depicting the systematic approach to anti-TB drug delivery system design. Chapter 1 of this study contains the introduction and the rationale for the research. The introduction concisely describes the current situation in terms of TB chemotherapy and provides a glimpse of novel and future trends in anti-TB drug delivery, emphasising the pertinent advantages of these forward strides but also stressing the need for a novel oral system that could overcome the bioavailability concerns of currently available fixed-dose anti-TB drug combinations in addition to assisting in achieving improved patient compliance with the existing regimen.

The second chapter of this study describes the considerations pertinent to the development of a dispersible multiparticulate system incorporating the water-soluble INH and poorly soluble RIF. Important concepts, such as site-specific delivery of multiparticulates and gastrointestinal physiology relative to an enteric-coating function, in addition to the requisites for a reconstitutable system, are put forth.

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Elucidation of the enteric-release principles applicable to the development of an enterosoluble system lead to systematic and pragmatic investigation of feasible methods for its fabrication in Chapter 3. A candidate formulation, possessing the advantages of simple and effective manufacture, and favourable *in vitro* release behaviour, was identified utilising a model-independent approach for further investigation and optimisation.

A Box-Behnken experimental design was employed to synthesise several variants of the candidate formulation, which were characterised in terms of their physicomechanical, drug entrapment and release properties. Identification of the optimum enterosoluble multiparticulate system instituting the principles of Response Surface Methodology, having appropriate INH entrapment and release characteristics forms the crux of the fourth chapter.

Chapter 5 seeks to further elaborate on the morphological and physicochemical characteristics and transitions of the enterosoluble system. Because there is an unequivocal relationship between the properties of a cross-linked enterosphere and its structure in such a way that both characteristics cannot be considered in an isolated way, and because the polymeric composition and synthesis method decisively influence the structure of the enterosphere as well as the final properties that the structure will have; in-depth analyses on drug-free and drug-loaded enterosoluble multiparticulates was systematically undertaken.

Chapter 6 discusses issues of pivotal importance in the development of a gel-forming suspending agent for adequate suspension of the INH-loaded enterosoluble system and poorly water-soluble RIF. Investigated extemporaneous hyrophilic polymeric gels having appropriate suspension capabilities are discussed. Such considerations allowed for the identication of a novel composite suspension system. With reference to the novelty of this system, the suspending agent has to be

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evaluated in terms of its ability to form an extemporaneous supporting network with tepid water capable of inhibiting settling or floating of the INH-loaded enterosoluble multiparticulates and the poorly soluble RIF. Progressive methods for the analytical characterisation of an identified suitable agent/s were initiated through appropriate physicomechanical analyses in the form of viscometric and textural tests.

The suspension system was further developed and evaluated for its stability in Chapter 7, with identication of the optimum system for inclusion in reconstitutable multiparticulates incorporating RIF. Elucidation of the optimum levels of each suspending and gel-forming agent was by a Face-Centred Central Composite Design.

The crux of this investigation was the attainment of segregated gastrointestinal delivery of RIF and INH in order to address issues of unacceptable RIF bioavailability on co-administration with INH. In Chapter 8, the proposed United States Pharmacopoeial (USP) high performance liquid chromatographic (HPLC) and colorimetric method, and appropriately applied regressional analysis of ultraviolet (UV) spectrophotometric absorbance data were employed for *in vitro* resolution of RIF and INH release at simulated gastric pH for comparison with the release profiles of FDCs commercially available in South Africa.

Chapter 9 addresses future nanotechnological trends in anti-TB drug delivery. A nanoparticulate-based drug delivery system was fabricated as an explorative extension of the novelly identified approach developed and optimised in Chapters 3 and 4. This system addresses issues of patient non-compliance, bioavailability and toxicity in TB treatment. The technology aims to reduce both the duration and frequency of treatment through effective, targeted and controlled release of anti-TB drugs.

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The final chapter concludes the dissertation and ties together the significant issues addressed regarding the formulation of an anti-TB drug delivery system, with recommendations for future investigations in terms of system applicability.

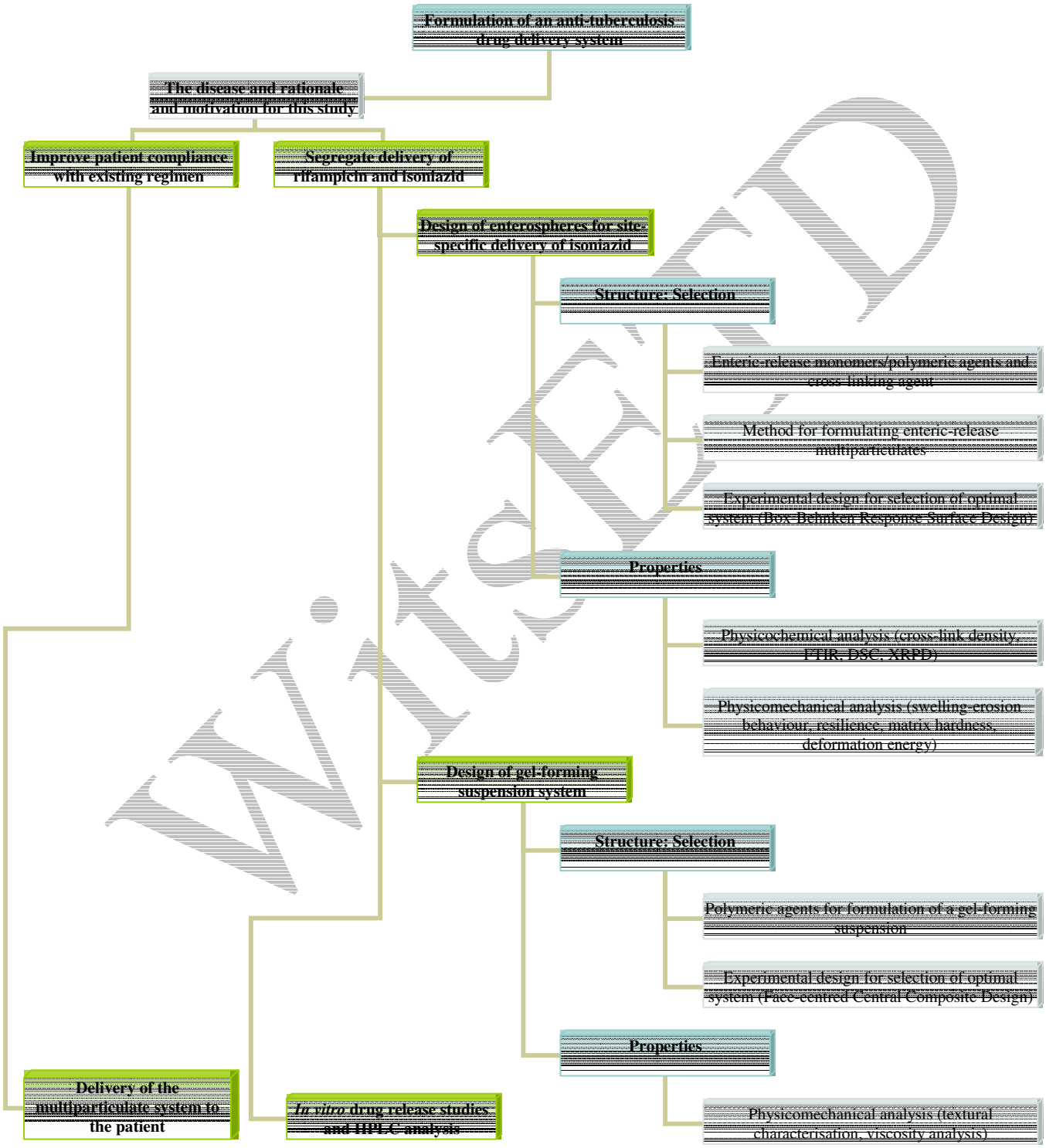

Figure 1.7: Organogram of a rational approach to the design of an anti-TB drug delivery system