Chapter 1

Introduction

“I seem like a boy playing on the sea shore & diverting myself in now and then finding a smoother pebble or prettier shell than the ordinary, whilst the great ocean of truth lay all undiscovered before me”

... Issac Newton
Introduction

1.1 Oral multiparticulate drug delivery system

Pharmaceutical oral solid dosage forms have been used widely for decades and oral route of drug administration is generally preferred because of its ease of administration and better patient compliance. The commonly used pharmaceutical oral solid dosage forms include granules, pellets, tablets and capsules (Rubinstein, 2000).

Oral dosage form can be broadly classified into two categories: Single-unit and Multiple-unit dosage forms. The single-unit dosage forms include matrix tablet or coated/uncoated tablet or capsules. The multiple-unit dosage forms consist of pellets or microencapsulated drug filled in a capsule or compressed into a tablet (Ghebre-Sellassie, 1989). The basic concept of multiple-unit systems is that the dose of the active ingredient is released by the individual subunits (like, pellets), and the functionality of the entire dose depends on the quality of the subunits.

Pellets are agglomerates of fine powders or granules of bulk drugs and excipients. Pellets consist of small, free-flowing, spherical or semi-spherical solid units, typically from about 0.5 mm to 1.5 mm, and are intended usually for oral administration (Kristensen and Schaefer, 1987, Ghebre-Sellassie, 1989). Pellets offer several advantages over a single unit dosage form. Some of the advantages with respect to formulation are (Ghebre-Sellassie, 1989; Melia et.al., 1994):

- Ease of handling, such as filling into capsules
- Different dosage strengths without formulation and process changes
- Incorporation of otherwise incompatible ingredients in a single dosage form
- Different release profiles at different sites in the gastrointestinal tract (GIT)
- Protection against degradation of active ingredients by oxidation or moisture by protective film coating
- High degree of patient acceptance when filled in capsules due to their elegance as compared to tablets
- Ideal shape for application of film coatings due to low surface to volume ratio
In addition, to the formulation advantages there are therapeutic advantages of pelletized dosage forms which are as follows:

- Minimal local irritation in the GIT
- Maximized drug absorption
- Lower risk of dose dumping
- Better reproducibility of therapeutic effects
- Reduced inter-and intra-subject variability

1.2 Methods of preparing pellets

Pellets have been known in the pharmaceutical industry for a long time. Some of the techniques available for the pellet manufacturing include (Ghebre-Sellassie, 1989; Melia et al., 1994)

- Extruder and spheronizer
- Fluid-bed layering
- Fluid-bed rotogranulator
- Coating pan

1.3 Extrusion-Spheronization

Extrusion/spheronization is one of the widely used pelletization process in the pharmaceutical industry. Extrusion and spheronization technology was developed for pharmaceutical applications in the early 1960s. Since then, it has gained popularity in pharmaceutical dosage form development (Ghebre-Sellassie, 1989).

Extrusion-spheronization is the process of converting powdered raw material into a product of uniform spherical units or pellets, under controlled conditions. The extrusion process comprises of forcing the wet plastic mass through a small orifice (extrusion die), thus forming cylinders or strands with a breadth corresponding to the die diameter and a length which depends on material properties and extruder type (Hicks and Freese, 1989). While, spheronization is the process whereby the cylindrical extrudates undergo a number of subtle shape changes, i.e., long strands to short uniform rods, short rods to rods with ellipsoids and to spheroids, when spheronized on a friction plate under controlled conditions (Sherrington and Oliver, 1981).
The pellets manufactured by extrusion and spheronization involve several steps as depicted in Fig 1. The drug and the excipients are blended and wet massed in a suitable mixer and then extruded. The resultant strands of extrudates are placed in the spheronizer, where these are broken into short cylindrical rods on contact with the rotating friction plate. Due to the centrifugal force, these rods are forced towards and up the stationary wall of the spheronizer which then fall back to the friction plate due to the gravity. This cycle is repeated until the desired spherical pellets are obtained (Rowe, 1985; Ghebre-Sellassie, 1989).

**Fig 1.** Flow chart depicting a typical extrusion-spheronization process

1.4 Theory of pellet formation and growth

The pelletization process is basically an agglomeration process that converts fine powder of drug and excipient into small, free-flowing, spherical units. Fine powder can be converted to agglomerates by the introduction of a liquid (aqueous/ nonaqueous) phase. The liquid and solid phase is brought into close contact by suitable agitation, leading to development of binding forces that causes agglomeration of powder. Growth of particles occurs either by collision and successful adherence of particles into discrete pellets or by the formation of nucleus onto which particles collide and attach themselves. This results in growth of particles. During growth phase the forces that hold the particles together include intermolecular attractive forces, electrostatic attractive forces and liquid bridges modes (Ghebre-Sellassie, 1989).
During pelletization, a uniformly blended powder mixture is granulated with a liquid like, water and the strength of the agglomerates depends on the liquid saturation level. The granulate strength can be additionally increased using more adhesive (viscous) binders. The wet mass densification occurs via extrusion and the resulting extrudates are brought together by capillary forces, mechanical interlocking (due to irregularities in particle shape), solid bridge formation (via solvent evaporation) and molecular forces (Ghebre-Sellassie, 1989). During spheronization process, moisture migrates towards the surface of the particles, thereby providing additional plasticity required for rounding of the pellets. Drying is the final phase where solvent is completely removed via evaporation and the pellet strength is mainly related to solid bridge formation (Wan, 1989).

1.5 Pellets as a controlled drug delivery system

Controlled release of therapeutic drugs is generally a preferred as it has the ability to localize delivery of the drug and maintain the concentration of a drug in the desired therapeutic range (Mehta et.al., 2009). Among the single unit and multiple unit oral controlled release dosage forms, multi-unit dosage forms have gained considerable popularity over conventional single units for controlled release technology. Pellets are frequently used in controlled-release systems because they are freely dispersed in the gastrointestinal tract and they offer flexibility for further modifications, such as coating (Kim et.al., 2007). Rapid and uniform dispersion of pellets in the gastrointestinal tract helps to maximize drug absorption, reduce peak plasma fluctuations, and minimize potential side effects without lowering drug bioavailability. They also reduce variations in gastric emptying rates and overall transit times. Thus, intra and intersubject variability of plasma profiles, which are common with single-unit regimens, are minimized. They are also less susceptible to dose dumping than the reservoir or matrix type, single-unit dosage forms (Ghebre-Sellassie, 1989). Other commonly reported advantage of pellets is that it is a suitable system for drug combinations especially when incompatibility between the drugs exist and release of the different drugs at different rates is required (Amighi et.al., 1998).

Oral multiparticulate (pellets) controlled release drug delivery system is thus advantageous over conventional delivery systems, particularly for long-term therapeutic effect and for the treatment of chronic diseases which require usage of multiple drugs.
Thus, there is a scope of using oral modified multiparticulate drug delivery system for the treatment of chronic disease like Tuberculosis (TB).

1.6 Tuberculosis

TB, a pervasive and deadly infectious bacterial disease, is one of the main challenges facing public health in developing countries (Sosnik et.al., 2009). It is an ancient disease and has taken a heavy toll of human life throughout the history of mankind. After 90’s, TB returned with vengeance and the global scourge of multi-drug resistant TB (MDR-TB) is reaching epidemic proportions. The burgeoning spread of drug resistant strains is worrisome and highly disturbing because the survival rates are almost negligible (WHO, 2009a).

TB has already victimized a large section of the world population and is still affecting many lives at an unmodified speed. Approximately, 1.8 billion people are currently infected with Mycobacterium tuberculosis (Mtb), representing about 30% of the global population (WHO, 2009b). More than 8 million people develop active TB every year, and approximately two million die annually. After acquired immunodeficiency syndrome (HIV/AIDS), TB is the world’s second most common cause of death from infectious disease. Over and above, HIV/AIDS has fuelled the spread of TB. TB is endemic in most of the developing countries and resurgent in developed and developing countries with high rates of human immunodeficiency virus (HIV/AIDS) infection (WHO, 2009a).

1.6.1 Pathogenesis of TB

In 1882, Robert Koch identified the tubercle bacillus, M. tuberculosis, as the cause of TB in humans. This pathogen is still known by many as “The Koch’s bacillus” (Panchagnula and Agrawal, 2004). M. tuberculosis is a highly virulent, airborne, slow-growing, gram-positive, aerobic, rod-shaped acid-fast bacillus. The cell wall of M. tuberculosis has high lipid content and helps the bacteria to survive within macrophages. It also provides the organism with a resistant barrier to many of the common drugs (Jawetz, 1982; Lamke, 2008). The World Health Organization (WHO) estimates that 1.8 billion people worldwide are infected by M. tuberculosis and most of them are clinically latent. The mechanism of this latency is poorly understood and is still a subject of investigation (Blasi et.al., 2009).
Man is the primary host for *M. tuberculosis*. TB infection is spread *via* airborne dissemination of aerosolised bacteria containing droplet nuclei of 1–5 μm in diameter from an infected individual to an uninfected individual (Sutherland, 1976). The bacteria are non-specifically phagocytosed by alveolar macrophages and their multiplication within macrophages is initiated (Smith, 2003). This is followed by the exponential increase in the number of pathogens by killing host cells and spreading locally to regional lymph nodes in the lungs by lymphatic circulation (3 to 8 weeks after infection). After the initial infection, intracellular replication of bacilli occurs, and dissemination of organisms may result through lymphatic and haematogenous routes (Matsushima, 2005).

Clinically, the main focus of TB infection is lungs. The prominent symptoms are chronic productive cough, low grade fever, night sweats, fatigue and weight loss (WHO, 2007). TB may present extra-pulmonary manifestations including lymphadenitis, kidney, bone, or joint involvement, meningitis or disseminated (miliary) disease (Matsushima, 2005). At this stage, acute TB meningitis or disseminated TB can sometimes result in death.

The frequency of such extra-pulmonary manifestations is increased among immune-compromised individuals such as in elderly, malnourished or HIV/AIDS individuals. Only 6 to 10% of HIV-negative patients develop the disease and, in most of the cases, because of the reactivation of a pre-existing infection. In contrast, HIV/AIDS patients have a 50 to 60% chance to show reactivation during a lifetime (Schluger, 2005).

### 1.6.2 Tuberculosis in the world of today

Even a century after Koch’s discovery of the tubercle bacillus and decades after the discovery of powerful anti-TB drugs, TB remains a leading cause of death in the developing world. In view of the severity and spread of the disease, in 1993, World Health Organization (WHO) declared TB to be a ‘global emergency’ with more than 1.9 million people infected (Fox, 1990a; Singh *et al.*, 2001). Globally, TB causes 2 million deaths per year. In 2008, there was an estimated 9.4 million (range, 8.9–9.9 million) incident cases (equivalent to 139 cases per 100,000 population) of TB globally (WHO, 2009).
TB is a disease of poverty affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world, and more than half of all deaths occur in Asia. China and India accounted for an estimated 35% of all undetected new smear-positive cases in 2008. Most of the estimated number of cases in 2008 occurred in Asia (55%) and Africa (30%), with small proportions of cases in the Eastern Mediterranean Region (7%), the European Region (5%) and the Region of the Americas (3%) (WHO, 2009). A global estimate of TB incidence rate, by country is shown in Fig 2. It is estimated that India accounts for one fourth of the global TB burden, with an estimated 14 million cases to which about 2 million are added every year, and an annual death toll of 500,000 people.

TB and HIV/AIDS form a lethal combination, each speeding the other's progress. TB control is jeopardised by the HIV epidemic. A third of the 40 million people living with HIV/AIDS are infected with *M tuberculosis*. In 2003, about 674,000 HIV-positive individuals developed tuberculosis, which represents the main cause of death in such individuals (Aziz *et al.*, 2006). The deadly synergy between TB and HIV has led to a quadrupling of TB cases in several African and Asian countries, and threatens to make TB incurable in the future (Cavenaghi, 1989; WHO, 2009).

Today, the situation is exacerbated by the dual epidemic of TB and human immunodeficiency virus (HIV) and spread of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) (WHO, 2009). There were 9.4 million new TB cases in 2008, (3.6 million of whom are women) including 1.4 million cases among people living with HIV (WHO, 2009). Multi Drug Resistant TB (MDR-TB) is a form of TB that is difficult and expensive to treat and patient fails to respond to standard first-line drugs. While, extremely drug resistant TB (XDR-TB) occurs when resistance of patient fails to second-line drugs.

Although current treatment can be effective if administered correctly, existing drugs must be taken for at least 6 months (M) to prevent relapsing disease. Low treatment compliance contributes directly to the emergence of MDR and XDR strains of *M. tuberculosis*, which further limit the efficacy of standard therapy (Sassetti and Rubin, 2007). The emergence of drug-resistant strains occurs with the wide use and misuse of antimicrobials (Aziz *et al.*, 2006).
Drug resistant TB represents a substantial challenge to tuberculosis control programmes, since the treatment of such cases is complex, more costly, and frequently less successful than treatment of non-resistant strains. Cure rates in cases harbouring MDR strains ranges from 6% to 59% (Espinal et al., 2000; Aziz et al., 2006).

In 2008, WHO released findings from its largest MDR-TB survey and reported the highest rates of MDR-TB ever recorded with peaks up to 22% of new TB cases in some settings of the former Soviet Union. In the same region, 1 in 10 cases of MDR-TB is XDR-TB (WHO, 2009). The WHO estimates that the number of new MDR-TB cases in 2004 was 425,000, with China, India, and the Russian Federation accounting for just over 60%. To reduce the global burden of TB, in 2006, WHO launched the new stop TB strategy. The core of this strategy is directly observed treatment shortcourse (DOTS), a TB control approach launched by WHO in 1995. However, collectively, these statistics show that TB remains a major global health problem (WHO, 2009).

The regimen for the treatment of MDR-TB include several potential antibiotics like amikacin, ofloxacin, ciprofloxacin, capreomycin etc, which are not essentially antitubercular agents and generally prove very toxic after long treatment duration (18M or more). Further such regimens are very costly, mostly beyond the reach of ordinary poor patients (Savale, 2003).
1.6.3 Treatment of TB

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 available option. 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., 1990b). WHO strongly recommends that treatment of active TB should not be attempted with a single drug. The treatment with single drug results in the development of MDR-TB (WHO, 1999). Anti-TB drugs with their daily recommended dose are enlisted in Table 1. Antitubercular drugs may be divided into two groups according to their clinical usefulness:

- First Line Agents: rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin and thioacetazone
- Second Line Agents: kanamycin, cycloserine, ethionamide and capreomycin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose in mg/Kg body weight</th>
<th>Duration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Ethambutol Hydrochloride</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>25</td>
<td>2</td>
</tr>
</tbody>
</table>

The first line and second line anti-TB drugs along with their potency are schematically represented in Fig 3. If organisms prove resistant and cannot be treated with an appropriate combination of drugs from the first line agents, less common and generally more toxic second line agents must be employed. As suggested by WHO (1999), treatment of TB and drug resistant case requires multi-drug therapy, given in two phases:

- Intensive phase comprising of rifampicin, isoniazid, pyrazinamide, ethambutol, daily for 2M,
- Continuation phase - rifampicin and isoniazid for a further 4M, either daily or 3 times a week.

Isoniazid eradicates most of the rapidly replicating bacilli in the first 2 weeks of treatment, together with ethambutol. Thereafter, rifampicin and pyrazinamide have an
important role in the sterilisation of lesions by eradicating organisms; these two drugs are crucial for successful 6 M treatment regimen (Ellard and Fourie, 1999). Rifampicin kills low or non-replicating organisms isoniazid and rifampicin, the two most potent anti-TB drugs, kill more than 99% of tubercule bacilli within 2 M of initiation of therapy (Mitchison, 1985; Iseman and Madsen, 1989). Using these drugs in conjunction with each other reduced the treatment period from 18 M to 6 M.

Fig 3. First line and second line anti-TB drugs (Dorman and Chaisson, 2007)

TB treatment is a multi-drug regimen and the use of combination therapy in a standardized regimen is the fundamental strategy of WHO and International Union against Tuberculosis and Lung Disease (IUATLD) for treatment of TB. However, with increase in the number of drugs to be taken, problem of patient compliance increases. “Combo-packs” for TB treatment (in which all the pills are to be taken at one time are packed together, to reduce the chances of a patient missing doses) were introduced in an attempt to solve this problem. However, even when using combo-packs patients can fail to take the drugs by choosing some and leaving out the others. Despite, the development of calendar packs, the problem of patient compliance persisted (Blomberg et.al., 2002).

Inconsistent or partial treatment, when patients do not take all their medicines regularly for the required period results in the development of MDR-TB. Thus, the concept of Fixed Dose Combination (FDC) came as a further step in the solution to this problem (Ellard and Fourie, 1999; Bloomberg, et.al., 2002).
1.6.4 Fixed Dose Combination (FDC) for the treatment of TB

One of the best ways of ensuring patient compliance with multi-drug regimens is to combine the requisite drugs physically into a combination preparation – a FDC product (Bloomberg et al., 2002). The rationale for using FDCs for TB stems from the fact that TB treatment always requires a multi-drug therapy (Panchagnula, 2001). WHO and IUATLD recommend the use of FDC formulations as routine practice in the treatment of TB. The FDCs have been included in the “List of Essential Drugs” issued by WHO (Amidon, 1995; Lobenberg and Amidon, 2000; Shishoo et al., 2001).

Anti-TB FDC formulations combine two or more first-line anti-TB drugs (namely rifampicin, isoniazid, pyrazinamide and ethambutol) in a fixed proportion in a single dosage form. There are two types FDC available for the treatment of TB, a four drug FDC of rifampicin, isoniazid, pyrazinamide and Ethambutol, which is given for the initial 2M and two drug FDC of rifampicin and isoniazid, which is given for the subsequent 4M (WHO, 2010). The potential advantages associated with the use of FDCs include (Bloomberg et al., 2002):

- Better patient compliance
- Safety and efficacy
- Simplified treatment
- Dosage adjustment according to individual need
- Better management of DOTS
- Simplified drug supply management, shipping and distribution
- Reduced risk of emergence of drug-resistant strains

1.6.5 Problems associated with anti-TB FDCs

WHO and IUATLD recommend the use of FDC formulations as routine practice in the treatment of TB. FDC formulations of anti-TB drugs have several distinct advantages over single drug formulations (Bloomberg et al., 2002). Therefore, extensive efforts have been made to promote them in TB therapy (WHO, 1999). However, serious concern has been raised on the utility of FDC products due to their quality problems.
Over the years, two major problems have been identified (Laserson et al., 2001; Shishoo et al., 2001; Immanuel et al., 2003; Singh and Mohan, 2003; Bhutani et al., 2004a; Luyen et al., 2005)

- Impaired and variable bio-availability of rifampicin when combined with isoniazid
- Instability of the rifampicin in FDC formulations containing isoniazid.

1.6.5.1 Impaired and variable bioavailability of rifampicin from the FDCs

The ‘bioavailability’ problem of FDCs was highlighted for the first time way back in 1989 by Acocella, who observed that one out of three FDCs containing rifampicin and isoniazid, and all the four FDCs containing rifampicin, isoniazid and pyrazinamide had significantly lower plasma concentrations of rifampicin.

It was observed that in normal adults the peak plasma concentration ($C_{\text{max}}$) after administration of 600mg rifampicin alone is in the range of 6-13µg/ml (Ellard and Fourie, 1999). However, administration of rifampicin along with isoniazid, and/or pyrazinamide as "separate formulations" (administered at the same time) or as FDCs, results in the $C_{\text{max}}$ values in the range of 3 to 6 µg/ml (Acocella, 1989). At least three independent studies have been reported (Shishoo et al., 2001; Immanuel et al., 2003; Luyen et al., 2005), wherein, FDCs were tested against rifampicin-alone formulations according to the Acocella’s approach. In all of them, an almost 30% fall in bio-availability of rifampicin was observed.

Rifampicin is the only sterilizing drug available and an important component of anti-TB therapy to be used for treatment of all categories of patients both in intensive and continuation phases. Hence, using FDC tablets with poor rifampicin bioavailability can lead directly to the treatment failure and may encourage drug resistance. Furthermore, clinical and bacteriological investigations have revealed that the anti- mycobacterial activity of rifampicin is dose-dependent (Panchagnula et al., 1999). Studies in literature indicate that a fall in the dose of rifampicin below 9 mg/kg leads to failure of therapy and can contribute towards development of the drug resistance (Long et al., 1979). Currently, the prescribed dose of rifampicin is 10 mg/kg, which means a narrow margin of only 10% between the actual delivered dose and the minimum necessary dose for therapeutic action. The drug has been reported to degrade in the presence of isoniazid, which means there exists a strong possibility of the dose of
rifampicin falling below the minimum required level, after administration of formulations containing the two drugs in combination (Sankar et al., 2003).

Much effort had been made by the WHO and other international agencies to address the bio-availability problem of rifampicin in FDCs (Kenyon et al., 1999; Laserson et al., 2001; Singh and Mohan 2003; Bhutani et al., 2004a; Bhutani et al., 2004b), from the time it was highlighted in 1989 (Acocella, 1989). The variable bioavailability of rifampicin from solid oral dosage forms has also been reported. In 1994, WHO and IUATLD sounded a warning that anti-tubercular FDC formulations should be used only if the bioavailability of rifampicin has been demonstrated convincingly. A protocol was published as a joint statement of IUTALD/WHO for testing bioequivalence of rifampicin from FDC products (IUTALD/ WHO, 1994).

However, at the same time, there are several simultaneously contradictory reports suggesting that there is no statistical difference in the oral bioavailability of rifampicin after administration of rifampicin along with isoniazid, thereby adding to the confusion. Many of these reports, however, are based on non-specific methods including microbiological methods (Shishoo et al., 2001).

In literature, rifampicin bioavailability has been reported to be multifactorial. The reasons hypothesized in the literature include raw material characteristics, changes in the crystalline habit of the rifampicin, excipients, manufacturing and/or process variables, degradation in the gastro-intestinal tract, inherent variability in absorption and metabolism, etc. (Laing et al., 1999; Bloomberg et al., 2002; Panchagnula and Agrawal, 2004; Singh et al., 2001). In the product development or manufacturing of FDCs, rifampicin is the only water-insoluble component and hence its incorporation with other highly water-soluble drugs is a critical process, which is further complicated by the number of processing steps commonly implicated in single-unit dosage form preparation, such as grinding, mixing, granulation, and drying that may alter the crystalline nature, particle size, dosage form characteristics and release behaviour thereby affecting its bioavailability (Bloomberg et al., 2002; Laing et al., 1999; Agrawal et al., 2004a; Agrawal et al., 2004b). In addition, common pharmaceutical excipients employed in tablet manufacture, such as binder and glidant may adversely affect rifampicin release through drug adsorption and subsequently reduce its gastrointestinal
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(GI) absorption. The effect of these factors, however, has not been as convincingly explained or demonstrated in previous studies (Panchagnula and Agrawal, 2004).

It is generally considered that the variable bioavailability of rifampicin was largely confined to FDC formulations; however, reduced plasma concentrations following administration of rifampicin only formulations has also been reported by Zak et.al., 1981 and McIlerson et.al., 2002.

Furthermore, an apparently satisfactory in vitro dissolution test did not ensure acceptable rifampicin bioavailability (IUTALD/WHO, 1994). The in vitro dissolution tests do not guarantee in-vivo bioavailability of rifampicin. It is reported that formulations showing poor dissolution had good bioavailability and vice versa (Shishoo et.al., 1999; Aspesi, 1989; Agrawal and Panchagnula, 2004).

**1.6.5.2 Instability of the rifampicin in FDC formulations**

The identification of ‘stability’ problem of rifampicin and isoniazid FDC is of relatively recent origin and was first highlighted by Laserson et.al., 2001. These workers found that anti-TB FDC products with lower than required strength of rifampicin were in wide circulation. The stability related problems include changes in drug strength, increase in degradation product levels, alteration in dissolution profile, gain in moisture, etc. The stability problem has been highlighted further in subsequent studies and has been found to be more acute with three or four-drug FDCs containing rifampicin and isoniazid, in comparison with formulations containing these two drugs (Singh et.al., 2002; Singh and Mohan, 2003; Bhutani et.al., 2005a). Several reports indicate that rifampicin and isoniazid in the solid dosage form degrade upto 22% and 32% respectively. This solid-solid interaction is accelerated by humidity, light and temperature (Bhutani et.al., 2004a; Bhutani et.al., 2004b). This signifies that there exists a strong possibility of falling of dose of rifampicin below the minimum therapeutically effective level of 10 mg/kg body weight.

Thus, apart from the initial drug content in formulations, stability of rifampicin in its dosage forms and under stomach acid conditions turns out to be an important factor in assuring therapeutic action of the drug (Sankar et.al., 2003).
1.6.6 Factors responsible for the impaired bioavailability and instability of rifampicin FDC formulation

Extensive research has been conducted by two independent groups in India in recent years, to decipher the problem of bioavailability and stability of FDCs. An elegant mechanism has been proposed to explain the reaction of rifampicin with isoniazid in the acidic medium of the stomach. Further, this reaction between rifampicin and isoniazid has also been reported in the solid dosage form (Singh et al., 2000a; Singh et al., 2000b; Shishoo et al., 2001; Singh et al., 2001; Sankar et al., 2003). The proposed mechanism for this reaction is shown in Fig 4.

Rifampicin is known to undergo hydrolysis in acidic medium to the insoluble 3-formyl rifamycin SV (3-FRSV). Isoniazid accelerates degradation of rifampicin into this poorly absorbed derivative (3-FRSV) in the acidic environment of the stomach via reversible formation of the isonicotinyl hydrazone (HYD) of 3-FRSV with isoniazid (Singh et al., 2000a; Singh et al., 2000b; Shishoo et al., 2001; Singh and Mohan, 2003). Earlier, Devani et al., 1985, has reported that isoniazid reacts with the reducing sugars (aldehyde/ketone) to form hydrazones in a reversible manner.

Shishoo et al., (2001) has indicated that rifampicin in the presence of isoniazid as a FDC may undergo greater decomposition in the gastric environment, as compared to when rifampicin is administered (orally) alone. Thus, less rifampicin will be available for absorption from FDCs as compared to rifampicin administered as a separate formulation. This will be reflected in the poor bioavailability from the former formulation. This reaction has been ascribed to be responsible for the reduction of in-vivo bioavailability of rifampicin from FDC products (Immanuel et al., 2003; Shishoo et al., 2001).

Interestingly, the bioavailability of isoniazid is not affected by the interaction between rifampicin and isoniazid. This has been explained to the reversible nature of the reaction between isoniazid and 3-FRSV, shown in Fig 4. The isonicotinyl hydrazone is converted back to isoniazid and 3- formylrifamycin, resulting in recovery of isoniazid, but eventually causing the loss of rifampicin due to formation of inactive hydrazone. This explains why the bio-availability problem is confined to rifampicin alone and not isoniazid (Singh et al., 2006). Also, concentration of isoniazid six times higher than the
rifampicin (isoniazid: rifampicin; 6:1 on the molar basis) in the FDC may also play a role (Savale, 2003).

Fig 4. Schematic representation of mechanism showing interaction of rifampicin and isoniazid in the acidic medium

Further studies have established that the reaction between rifampicin and isoniazid to hydrazone occurs even in the solid formulation environment. This was found when FDC products were exposed to accelerated stability test conditions of temperature and humidity (Singh and Mohan, 2003; Bhutani, et. al., 2005b). Stability of FDC formulations at high temperature and humidity is a matter serious for tropical countries like, India, Africa.

The problem of interaction of rifampicin and isoniazid is compounded by pyrazinamide and ethambutol hydrochloride, the two co-drugs present usually in FDCs, by accelerating the reaction between rifampicin and isoniazid (Bhutani et. al., 2005a and b). It is postulated that pyrazinamide and ethambutol hydrochloride exhibit a catalytic role through involvement of intra-molecular proton transfer during reaction between rifampicin with isoniazid, which is conceived to occur through a base-catalyzed transhydrazone formation process entailing a tetrahedral mechanism (Bhutani et. al., 2005b). This explains the stronger physical and chemical changes in three- or four-drug FDCs, compared to that seen with those containing just the two drugs (Bhutani et. al., 2003; Singh and Mohan, 2003; Bhutani et. al., 2005 b).
1.7 Rationale of developing the novel FDC of rifampicin and isoniazid

The problem of reduced bioavailability of rifampicin from FDC products of anti-tuberculosis drugs is a matter of serious concern. An integral part of the strategy to fight the disease is the use of quality anti-TB drugs. The deficiency in delivery of proper dose of rifampicin has serious implications as it is known that doses of rifampicin less than 9mg/kg body weight can result in therapeutic failure (Long et.al., 1979) and hence can result in the development of drug resistance. The problems associated with quality of FDC products are in the current focus. The two major problems associated with the quality of FDCs are (i) loss of bioavailability of rifampicin upon administration, (Immanuel et.al., 2003; Shishoo et.al., 2001) and, (ii) instability of drugs within the formulation environment (Singh and Mohan, 2003; Bhutani et.al., 2005b). In both cases, the problem has been ascribed to the decomposition of rifampicin in the presence of isoniazid to form isonicotinyl hydrazone (Singh et.al., 2000a; Shishho et.al., 2001).

The decomposition of rifampicin both, in-vivo and in solid dosage form may culminate in the reduction in the dose from 10 to 12 mg/kg to as low as 5–6 mg/kg of body weight. A decrease in the dose of rifampicin below 9 mg/kg of body weight results in loss of therapeutic efficacy (Long et.al., 1979).

Hence, there is a critical need to redesign the current FDC formulation containing rifampicin and isoniazid. FDC products containing the two drugs need to be designed in such a manner that chances of interaction between them are reduced to the minimum under stomach acid conditions and in the formulation environment.

It has been reported that rifampicin shows a pH-dependant solubility, which affects its absorption from the GI tract (Savale, 2003). Rifampicin shows maximum solubility between pH 1-2 and is well absorbed from the stomach at this pH. This indicates that stomach is the site of optimum absorption of rifampicin. While, isoniazid is permeated less through the stomach and is mainly absorbed through the intestine (almost 60%). Isoniazid is poorly absorbed from the stomach because of the presence of its protonated form at acidic pH. However, it is well absorbed from all the three segments of the intestine (Mariappan and Singh, 2003).
Based on these observations, FDC product, devoid of both ‘bioavailability‘ and ‘stability’ problems, can be formulated by releasing and retaining rifampicin in the stomach and delivering isoniazid from the same formulation 3-4 h later in the intestine. Also, physical isolation of rifampicin and isoniazid within the FDC delivery system will improve drug stability during storage.

1.8 Formulation Design

The objective of the present study is to improve bioavailability and stability of rifampicin-isoniazid FDC formulation. A solution to prevent in situ loss and eventual decrease in rifampicin bioavailability from a FDCs lies in the redesigning of current FDC products containing rifampicin and isoniazid in such a way that the two drugs are released in different segments of the GIT. Rifampicin designed to be released in the stomach and isoniazid in the small intestine (through development of an enteric-release system), thus target them to their respective absorption windows (Mariappan and Singh, 2003). This strategy would also preclude physical interaction of these drugs within the dosage form during storage.

In view of this, the proposed formulation was designed to incorporate the following components of anti-TB FDC in a capsule:

- **Rifampicin**: Total dose of rifampicin was subdivided into two components
  - (i) Immediate release pellets of rifampicin- Loading dose of rifampicin
  - (ii) Gastroretentive floating pellets of rifampicin- Maintenance dose of rifampicin

- **Isoniazid**: Delayed release pellets of isoniazid

The proposed formulation will release the two drugs in a controlled manner with rifampicin being released in the stomach and isoniazid in the intestine. A schematic representation of the proposed formulation design of novel anti-TB FDC formulation is shown in Fig. 5.

From the proposed FDC formulation, it is expected that the rifampicin will be released immediately in stomach followed by its sustained release via gastroretentive floating formulation. It is expected that the immediate release formulation of rifampicin will provide the loading of the dose of rifampicin within its therapeutic window. While, the gastroretentive drug delivery system (GRDDS) of rifampicin will help to maintain the
concentration of rifampicin within its therapeutic window, at its site of absorption maxima i.e., stomach, for a prolonged period of time.

**Fig 5.** Schematic representation of the proposed formulation design of novel anti-TB FDC formulation

GRDDS are the formulations retained in the stomach for a prolonged period of time and release the drug in a controlled fashion (Moes, 1993; Singh and Kim, 2000; Whitehead *et.al.*, 1998). Prolonged release of rifampicin holds promise for reducing the dosing frequency and improving patient compliance, in the management of tuberculosis. Furthermore, as the gastric residence time of the formulation is extended, such system will reduce the frequency of administration and, thus, improved patient compliance (Stithit *et.al.*, 1998). GRDDS may be broadly classified into:

- High-density (sinking) systems
- Low-density (floating) systems
- Expandable systems
- Superporous hydrogel systems
- Mucoadhesive systems
- Magnetic systems

The proposed novel formulation of rifampicin will be based on floating drug delivery system (FDDS) concept. FDDS has a bulk density lower than the gastric content. They remain buoyant in the stomach for a prolonged period of time, with the potential for continuous release of drug. Eventually, the residual system is emptied from the stomach
(Singh and Kim, 2000). A FDDS formulation can be single unit (tablet/capsule) or multiple unit (pellets or granules) and can be further classified into-

- Hydrodynamically balanced systems
- Gas-generating systems
- Raft-forming systems
- Low-density systems

On the other hand, isoniazid release will follow delayed release pattern targeting it to its absorption maxima site, i.e. intestine. This release pattern would thus enable to segregate the release site of rifampicin and isoniazid and thus evade the *in-vivo* interaction among the two drugs.

In developing an oral system for anti-TB drugs, cognisance was also taken of the increase in popularity of multiparticulate (or multi-unit) solid dosage forms (e.g., beads, pellets, granules) in the area of oral controlled drug delivery. It is expected that such systems will be particularly useful for site specific targeting within the GIT. In view of bioavailability concerns, formulation of an anti-TB dosage form as an oral multiparticulate drug delivery system would also furnish many biopharmaceutical advantages when compared with solid single-unit dosage forms (Melia *et al.*, 1994).
Objectives of the Study

“A fact is a simple statement that everyone believes. It is innocent, unless found guilty. A hypothesis is a novel suggestion that no one wants to believe. It is guilty, until found effective”

........Edward Teller
1.9 Objectives of the study

The goal of this study was designing anti-TB drug delivery system, with improved oral effectiveness of the principle anti-TB agents, rifampicin and isoniazid. With drug bioavailability concerns in mind, the investigation is sought to attain this goal from the perspective of creating an efficient novel oral dosage form of rifampicin-isoniazid FDC. Specifically, the present study had the following well defined objectives:

1. To develop a novel site-specific FDC of rifampicin and isoniazid with a view to minimize degradation of rifampicin in the acidic medium and, to target the release of rifampicin and isoniazid at the site of their maximum absorption.

2. To develop formulation of rifampicin, both immediate release delivery system (loading dose) and gastroretentive floating delivery system (maintenance dose), and evaluate for their physico-chemical and release characteristics.

3. To evaluate the in-vivo gastric residence time of gastroretentive floating formulation using Gamma-scintigraphy.

4. To assess the stability of the developed rifampicin formulations, both immediate release delivery system (loading dose) and gastroretentive floating delivery system at room temperature and at accelerated stability conditions.

5. To develop the isoniazid delayed release delivery system and evaluate its physico-chemical and release characteristics.

6. To assess the stability of the developed delayed release isoniazid formulation, both at room temperature and at accelerated stability conditions.

7. To prepare rifampicin-isoniazid FDC and evaluate its stability, both at room temperature and at the accelerated stability conditions.

8. To assess the bioavailability of rifampicin and isoniazid from the developed novel FDC of rifampicin and isoniazid in healthy human volunteers.
References


