Oral Modified Multiparticulate Drug Delivery System for the Treatment of Tuberculosis

A Thesis Submitted to

NIRMA UNIVERSITY, Ahmedabad

For the Degree of

Doctor of Philosophy

In

PHARMACY

By

Amita K. Joshi

Under the Supervision of

Dr. Manish Nivsarkar

B. V. Patel Pharmaceutical Education
& Research Development (PERD) Centre, Ahmedabad

April, 2010

Certificate

This is to certify that the contents of this thesis entitled '**Oral modified multiparticulate drug delivery system for the treatment of tuberculosis**' is the original research work of **Ms. Amita Joshi** carried out under my supervision.

I further certify that the work has not been submitted either partly or fully to any other university or body –in quest of a degree, diploma or any other kind of academic award.

Date:

Place: Ahmedabad

Guide: **Dr. Manish Nivsarkar**
Joint Director and Head,
Dept. of Pharmacology &
Toxicology

Candidate's Statement

The work included in this thesis entitled '**Oral modified multiparticulate drug delivery system for the treatment of tuberculosis**' is an independent investigation carried out by me under the guidance and supervision of **Dr**. **Manish Nivsarkar**. In the thesis, references of the work done by others which have been used are cited at appropriate places.

I hereby declare that the work incorporated in the present thesis is original and has not been submitted to any other university or body – in quest of a degree, diploma or any other kind of academic award.

Date:

Place: Ahmedabad

Amita K. Joshi
(Reg. No. 05EXTPHDP07)

Acknowledgments

God is our refuge and strength, a very present help, when we need it most, I thank him for guiding me through rough and torn paths, for always being less than a whisper away…..I thank the almighty God, for it is under His grace that we live, learn and flourish !!

This thesis is the result of four years of work during which I have been accompanied and supported by many people. This thesis is also the result of many experiences I have encountered at PERD centre from dozens of remarkable individuals who I also wish to acknowledge.

Language is the most eloquent vehicle of human expression, yet on occasions one finds it difficult to get appropriate words to express the feelings fully. Speech fails me and I fumble for words to express my heart felt gratitude and sincere thanks to my worthy guide, **Dr. Manish Nivsarkar,** Joint director & Head, Department of Pharmacology & Toxicology, B.V. Patel PERD Centre, whose continuous encouragement, invaluable guidance, constructive criticism, precious suggestions, crucial help and benevolent attention, enabled me to execute this project successfully. I am indebted to him more than he knows.

With great pleasure and reverence, I express my debt of profound gratitude to **Prof. C. J. Shishoo**, Hon. Director, PERD Centre, for his supervision, advice, and guidance from the very early stage of this research as well as giving me extraordinary experiences throughout the work. His conceptual and technical insights into my thesis work have been invaluable.

I would also like to record my sincerest gratitude to **Prof. Harish Padh**, Director, B. V. Patel PERD Centre, for providing the most congenial working atmosphere and excellent laboratory facility in the centre. He provided unflinching encouragement and support in various ways. His distinct scientific intuition has made him a constant oasis of ideas and passion in science, which exceptionally inspire and enrich my growth as a student, a researcher and a scientist want to be. His constructive feedback and comments at various stages have been significantly useful in shaping the thesis upto completion.

I express my personal thanks & appreciation to **Dr. Kamala K. Vasu**, for her encouragement, support and loving gestures throughout the tenure of my research work.

It gives me immense pleasure to express my sincere thanks to **Dr. Neeta Srivastava** for her benevolent help and genuine concern.

I convey special acknowledgement to **Ashwini mam** and **Rajeshwari mam**, for their indispensable help and advice during bioanalytical studies, which was very fruitful in shaping up this project.

I sincerely thank **Prof R.S.R. Murthy** and **Prof Avani Amin**, for serving on my doctoral committee and for their constructive feedback and comments.

I take this opportunity to sincerely acknowledge **Council for Scientific and Industrial Research (CSIR)**, Govt. of India, New Delhi, for financial assistance in the form of SRF.

Gift samples of Rifampicin and Isoniazid by Cadila Pharmaceuticals Limited, Ahmedabad and Acryleze and HPMC by Colorcon India, Mumbai, are gratefully acknowledged.

I gratefully acknowledge the help of **Dr. Manas Mayank, Dr. Neelam, Mr. Omprakash** and staff of GCRI, Ahmedabad, for their zestful assistance to carry out the Gamma-Scintigraphic study.

I specially acknowledge the contributions of **Mr. Vishwas, Yogesh, Sumit, Umesh, Baljinder, Pravin, Meenu and Swati** for their help to carry out the Gamma-Scintigraphic study.

I specially thank **Sweta**, **Rita, Rakesh, Nitesh, Mehul** and **Nirav** for their immense help and support during human bioavailability study and its data processing.

From my first day in PERD, **Swati** and I have shared so many special moments, contributing to a common story whose colourful pages now lead me towards a new chapter. I am thankful to Swati, who helped me as and when required without giving second thought. She was always ready to extend all possible help, which gave me great encouragement. She has been an invaluable support, guide and more than a friend. She is my asset and the place to be solaced.

The warm support of all my friends at PERD enabled me to complete this thesis and have a wonderful time along the way. I need not thank my most cherished friends **Dr. Aryamitra Banerjee**, **Dr. Yogesh, Dr. Jitendra, Dr. Laxmi and Sundari**. Their motivation has helped me sail through all tough times.

I also debt my gratitude to **Thiru and Ranjeet** for sparing their time for me even at night during bioanalysis and taking care of my son.

It would be unfair if I don't mention my friends, **Sumit, Meenu, Umesh, Gopi, Balli and Pravin** for making this time memorable, with lots of cherished reminiscences to look back.

Special thanks to **Ranjeet** and **Sandeep** for proof reading my thesis manuscript.

I acknowledge **Vinayak Bhai** for his zestful association and good wishes. I also thank **Amrut Bhai** for his assistance. A word of thanks for the laboratory and technical staff: especially **Trivedi Bhai**, **Dinesh Bhai Prakash Bhai, Jasani Bhai** & **Kuralal Bhai,** for their cooperation and help.

I owe so much thanks to my **Parents** & **Parents in law** who have always supported, encouraged, and believed in me, in all my endeavours and who so lovingly and unselfishly cared for my son, Ayushmaan.

One of the most important persons who has been with me in every moment of my PhD tenure is my husband **Keertimaan**, for his steadfast support, patience & assistance in every step of my thesis. I would also like to thank to my son, **Ayushmaan** who joined us before this dissertation was complete, for giving me unlimited happiness, pleasure and bring smile when feeling low; yet, I am sorry for the time, we spent apart.

I would like to thank everybody who were important to the successful realization of this thesis, as well as express my apology that, I could not mention personally one by one.

Finally, when recounting a series of adventures, Satchel Paige was known to say, "Them were tall times." This phrase aptly expresses the sheer scale of the excitement, satisfaction and rich rewards this project has brought me. Tall times, Indeed !!

Amita Joshi

To my family ……

for being there with me all through !!

Table of contents

List of Tables

List of Figures

List of Abbreviations

25-DAR	25-Desacetyl Rifampicin
3D	Three Dimmensional
3-FRSV	3-Formyl Rifamycin SV
ACP	Acyl Carrier Protein
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
Anti TB	Anti-tubercular
Alu.	Aluminium
AR	Aspect Ratio
AUC	Area Under Curve
BCG	Bacillus Calmette-Guérin
BCS	Biopharmaceutical Classification System
BMI	Body Mass Indices
CAP	Cellulose Acetate Phthalate
CAT	Cellulose Acetate Trimellitate
CI	Confidence Interwal
C_{max}	Maximum Plasma Concentration
CNS	Central Nervous System
CO_2	Carbon dioxide
CRDF	Controlled Release Dosage Forms
DDS	Drug Delivery System
DE	Dissolution Efficiency
DMSO	Dimethyl Sulphoxide
DOTS	Directly Observed Treatment Shortcourse
DSC	Differential Scanning Calorimeter
25-DAR	25-Desacetyl Rifampicin
DSC	Differential Scanning Calorimeter
f_2	Similarity factor
FDC	Fixed Dose Combination
FDDS	Floating drug delivery system
FFD	Full Factorial Design
GI	Gastrointestinal
GIT	Gastrointestinal Tract
GRDDS	Gastroretentive Drug Delivery System

HBS	Hydrodynamically Balanced System
HCl	Hydrochloric acid
HDPE	High Density Polyethylene
HEC	Hydroxyethylcellulose
HIV	Human Immunodeficiency Virus
HPC	Hydroxypropylcellulose
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropylmethylcellulose
HPMCP	Hydroxypropyl Methylcellulose Phthalate
HYD	Isonicotinyl Hydrazone
ICH	International Conference on Harmonization
IUTALD	International Union against Tuberculosis and Lung Disease
K_{el}	Elimination rate constant
LOD	Loss on Drying
LQC	Limit of Quantification
LSM	Least Square Mean
MCC	Microcrystalline Cellulose
MIC	Minimum Inhibitory Concentration
MDR-TB	Multidrug Resistant Tuberculosis
MIC	Minimum Inhibitory Concentration
min.	Minutes
M	Months
Mtb	*Mycobacterium tuberculosis*
N	Normal
NaCMC	Sodium Carboxymethylcellulose
$NaHCO_3$	Sodium bicarbonate
NADH	Nicotinamide Adenine Dinucleotide
NLT	Not Less Than
NMT	Not More Than
NTPs	National Tuberculosis Programs
PE	Prediction Error
PEG	Polyethylene Glycol
PVP	Polyvinyl Pyrrolidone
REL counts	Relative counts
RH	Relative Humidity
rpm	Revloutions Per Minute

ROI	Region of Interest
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope
SSG	Sodium Starch Glycolate
$T_{1/2}$	Half life
TB	Tuberculosis
Tc	Technitium
T_{max}	Time corresponding to Maximum Plasma Concentration
WHO	World Health Organization
XDR-TB	Extremely Drug Resistant Tuberculosis

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

1

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 Schaefar, 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

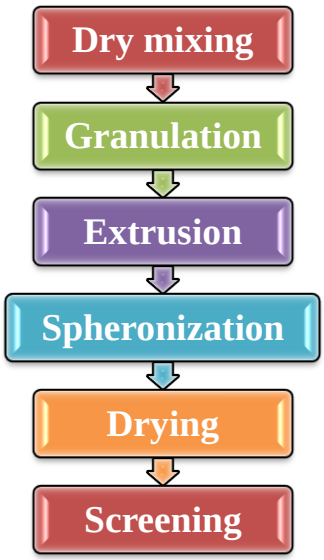
1.3 Extrusion-Spheronization

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).

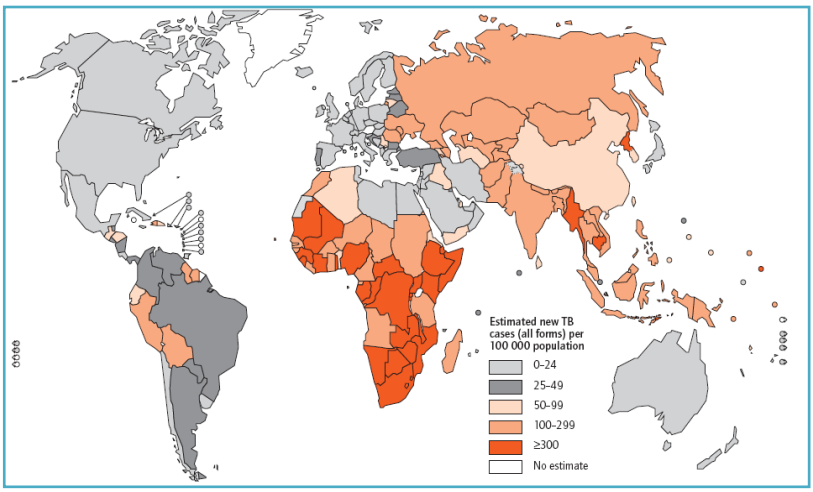
TB is a disease of poverty affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world, and more than half of all deaths occur in Asia. China and India accounted for an estimated 35% of all undetected new smear-positive cases in 2008. Most 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).

Fig 2. Estimated global TB incidence rates, by country, 2008 (WHO, 2009)



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:

a). First Line Agents- rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin and thioacetazone

b).Second Line Agents- kanamycin, cycloserine, ethinoamide and capreomycin.

Table 1. List of essential TB drugs with their recommended daily dose (WHO, 2010)

Drug	Dose in mg/Kg body weight	Duration (M)
Rifampicin	10	6
Isoniazid	5	6
Ethambutol Hydrochloride	15	2
Pyrazinamide	25	2

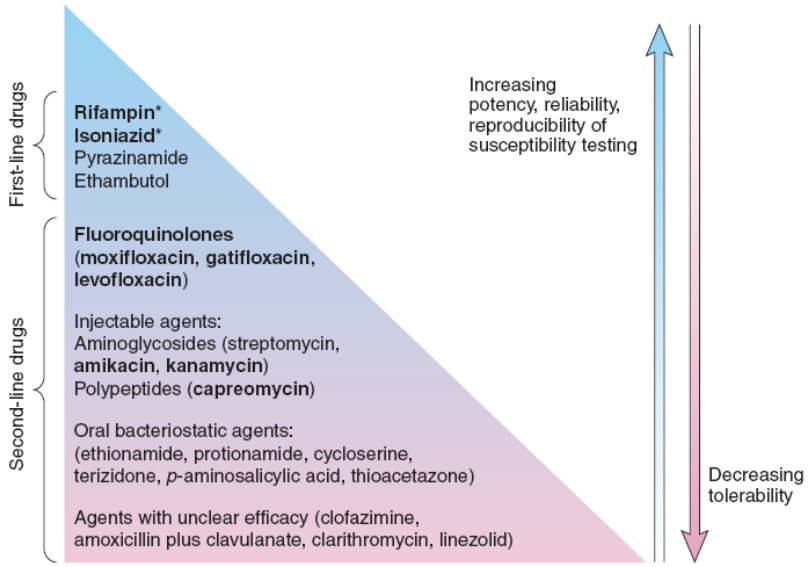
The first line and second line anti-TB drugs along with their potency are schematically represented in Fig 3. If organisms prove resistant and cannot be treated with an appropriate combination of drugs from the first line agents, less common and generally more toxic second line agents must be employed. 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_{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 pyrizinamide as "separate formulations" (administered at the same time) or as FDCs, results in the C_{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

(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 McIlleron *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.*, 2004 a; 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.

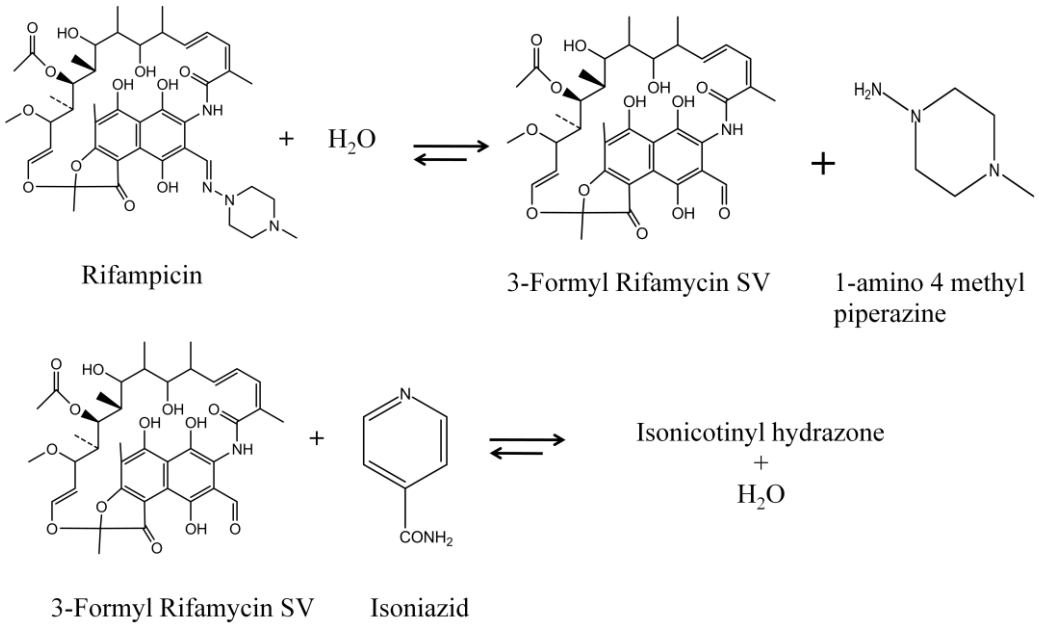
Rifampicin is known to undergo hydrolysis in acidic medium to the insoluble 3-formyl rifamycin SV (3-FRSV). Isoniazid accelerates degradation of rifampicin into this poorly absorbed derivative (3-FRSV) in the acidic environment of the stomach *via* reversible formation of the isonicotinyl hydrazone (HYD) 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; Shishoo *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:

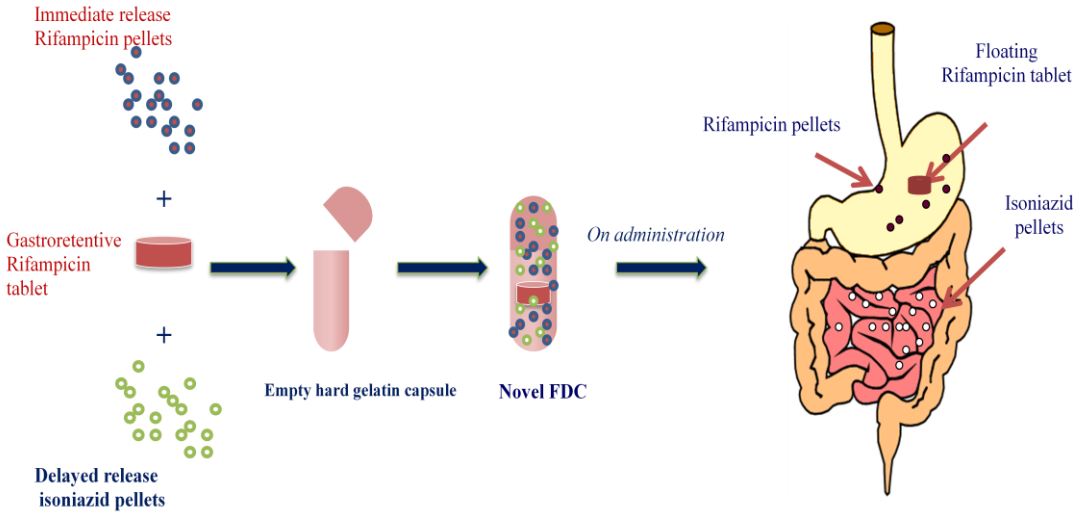
- **Rifampicin:** Total dose of rifampicin was subdivided into two components
  - (i) Immediate release pellets of rifampicin- Loading dose of rifampicin
  - (ii) Gastroretentive floating pellets of rifampicin- Maintenance dose of rifampicin
- **Isoniazid:** Delayed release pellets of isoniazid

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

Acocella, G., 1989. Human bioavailability studies. IUATLD Symposium. Quality control of anti-tuberculosis drugs. Bull. Int. Union Tuberc. Lung Dis. 64, 38-40.

Agrawal, S., Panchagnula, R., 2004. Dissolution test as a surrogate for quality evaluation of rifampicin containing fixed dose combination formulations. Int. J. Pharm. 287, 97–112.

Agrawal, S., Singh, I., Kaur, J.K., Bhade, S., Kaul, C.L., Panchagnula, R., 2004a. Bioequivalence trials of rifampicin containing formulations: Extrinsic and intrinsic factors in the absorption of rifampicin. Pharm. Res. 50, 317–327.

Agrawal, S., Ashokraj, Y., Bharatam, P.V., Pillai, O., Panchagnula, R., 2004b. Solid-state characterization of rifampicin samples and its biopharmaceutic relevance. Eur. J. Pharm. Sci. 22, 127–144.

Amidon, G.L, Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm. Res. 12, 413-420.

Amighi, K., Timmermans, J., Puigdevall, J., Baltes, E., Moes, A.J., 1998. Peroral sustained-release film-coated pellets as a means to overcome physicochemical and biological drug-related problems. Part 1. *In vitro* development and evaluation. Drug Dev. Ind. Pharm. 24, 509–515.

Aspesi, F., 1989. Dissolution testing. IUATLD Symposium. Quality control of anti-tuberculosis drugs. Bull. Int. Union Tuberc. Lung Dis. 4, 37-38.

Aziz, M.A., Wright, A., Laszlo, A., Muynck, A.D., Portaels, F., Deun, A.V., Wells, C., Nunn, P., Blanc, L., Raviglione, M., 2006. Epidemiology of antituberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. Lancet 368, 2142–54.

Bhutani, H., Mariappan, T.T., Singh, S., 2003. Behaviour of uptake of moisture by drugs and excipients under accelerated conditions of temperature and humidity in the absence and the presence of light. Part II. Packaged and unpackaged anti-tuberculosis products. Pharm. Technol. 27, 44-45.

Bhutani, H., Mariappan, T.T., Singh, S., 2004 a. A study on the physical and chemical stability of anti-tuberculosis fixed-dose combination (FDC) products under accelerated climatic conditions. Int. J. Tuberc. Lung Dis. 8, 1073-1080.

Bhutani, H., Mariappan, T.T., Singh, S., 2004b. An explanation for the physical instability of a marketed fixed dose combination (FDC) formulation containing Isoniazid and ethambutol and the proposed solutions. Drug Dev. Ind. Pharm. 30, 667-672.

Bhutani, H., Singh, S., Jindal, K.C., 2005a. Drug-drug interaction studies on first-line anti-tuberculosis drugs. Pharm. Dev. Technol.10, 517-523.

Bhutani, H.,Singh, S., Jindal, K.C., Chakraborti A.K., 2005 b. Mechanistic explanation to the catalysis by pyrazinamide and ethambutol of reaction between rifampicin and isoniazid in anti-TB FDCs. J. Pharm. Biomed. Anal. 39, 892–899.

Blasi, P., Schoubben, A., Giovagnoli, S., Rossi, C., Ricci, M., 2009. Fighting tuberculosis: old drugs, new formulations. Expert Opin. Drug Deliv. 6(9), 977-993.

Blomberg, B., Evans, P., Phanouvong, S., Nunn, P., 2002. Informal consultation on 4-drug fixed-dose combinations (4FDCs) compliant with the WHO model list of essential drugs. Geneva: World Health Organization. WHO/CDS/TB/2002.299.

Cavenaghi, R., 1989. Rifampicin raw material characteristics and their effect on bioavailability. Bull. Int. Union Tuberc. Lung Dis. 64, 36-37.

Dekker, T.G., Lotter, A.P., 2003. Anti-tuberculosis 4 FDC tablets mystery to chemistry. Int. J. Tuberc. Lung Dis.7, 205-206.

Devani, M.B., Shishoo, C.J., Doshi, K.J., Patel, H.B., 1985, Kinetic studies on the interaction between isoniazid and reducing sugars. J. Pharm. Sci., 74, 427.

Dorman, S.E., Chaisson, R.E., 2007. From magic bullets back to the magic mountain: the rise of extensively drug-resistant tuberculosis. Nature Med. 13(3). (http://www.nature.com/naturemedicine).

Ellard, G.A., Fourie, P.B., 1999. Rifampicin bioavailability: a review of its pharmacology and the chemotherapeutic necessity for ensuring optimal absorption. Int. J. Tuberc. Lung Dis., 3(11), S301–S308.

Espinal, M.A., Kim, S.J., Suarez, P.G., Kam, K.M., Khomenko, A.G., Migliori, G.B., Baez, J., Kochi, A., Dye, C., Raviglione, M.C., 2000. Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. JAMA 283, 2537–45.

Fox W., 1990a. Tuberculosis in India, past, present and future. Indian J. Tuberc. 37, 175-213.

Fox, W., 1990b. Drug combinations and the bioavailability of rifampicin. Tubercle. 71, 241-245.

Ghebre-Sellassie, I., 1989. Pellets: A general overview. In: Ghebre-Sellassie, I. (Ed.), Pharmaceutical Pelletization technology. Marcel Dekker, Inc., New York, USA, vol. 37, pp. 1-13.

Hicks, D.C., Freese, H.L., 1989. Extrusion and spheronizing equipment. In: Ghebre- Sellassie, I. (Ed.), Pharmaceutical Pelletization Technology, Marcel Dekker, Inc., New York, pp. 71-100.

Immanuel, C., Gurumurthy, P., Ramachandran, G., Venkatesan, P., Chandrasekaran, V., Prabhakar, P., 2003. Bio-availability of Rifampicin following concomitant administration of Ethambutol or Isoniazid or Pyrazinamide or a combination of the three drugs. Indian J. Med. Res. 118, 109-114.

Iseman, M.D., Madsen, L.A., 1989. Drug-resistant tuberculosis**.** Clin. Chest Med. 10, 341–53.

IUTALD/WHO, 1994. The promise and reality of fixed dose combinations with rifampicin. A joint statement of international union against Tuberculosis and Lung diseases and tuberculosis programme of the WHO. Tuber. Lung Dis. 75, 180-181.

IUTALD/ WHO A joint statement, 1999. Assuring bioavailability of fixed-dose combinations of anti-tuberculosis medications. Int. J. Tuber. Lung Dis. 3(11), S282–S283.

Jawetz, E., 1982. Antimycobacterial drugs. In: Katzung, B.G. (Ed.), Basic & Clinical Pharmacology. Lange Medical Publications, California, USA, pp. 503-519.

Kenyon, T.A., Kenyon, A.S., Kagarebe, B.V., Mothibedi, D., Binkin, N.J., Layloff, T.P., 1999. Detection of substandard fixed-dose combinations tuberculosis drugs using thin-layer chromatography. Int. J. Tuber. Lung Dis. 3(S): S347-S350.

Kim, M.S., Kim, J.S., You, Y.H., Park,H.J., Lee, S., Park, J.S., Woo, J.S., Hwang, S.J., 2007. Development and optimization of a novel oral controlled delivery system for tamsulosin hydrochloride using response surface methodology. Int. J. Pharm. 341, 97-104.

Kristensen, H.G., Schaefer, T., 1987. Granulation. A review of pharmaceutical wet granulation. Drug Dev. Ind. Pharm. 13, 803-872.

Laing, R., Fourie, B., Ellard, G., Sesay, M., Spinaci, S., Blomberg, B., Bryant, D., 1999. Fixed-dose combination tablets for the treatment of tuberculosis. Report of an informal meeting held in Geneva, World Health Organization, Geneva, WHO/CDS/CPC/TB/99.267.

Laserson, K.F., Kenyon, A.S., Kenyon, T.A., Layloff, T., Binkin, N.J., 2001. Substandard tuberculosis drugs on the global market and their simple detection. Int. J. Tuber. Lung Dis. 5, 448-454.

Lemke, T.L., 2008. Antimycobacterial agents. In: Williams, D.A., Roche, V.F., Zito, S.W. (Eds.) Foye's Principles of Medicinal Chemistry. Lippincott, Williams and Wilkins, Philadelphia, USA, 6th ed., pp. 1127-1146.

Lobenberg, R., Amidon, G.L., 2000. Modern bioavailability and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur. J. Pharm. Biopharm. 50, 3-12.

Long, M.W., Snider, D.R., Farer, L.S., 1979. US Public Health Service Cooperative Trial of Three RIF INH Regimens in the treatment of pulmonary tuberculosis. Am. Rev. Respir. Dis. 1979, 119, 879–894.

Luyen, L.T., Huyen, H.T.K., Sang, T.V., Huong, N.T.L., 2005. Effect of Isoniazid and Pyrazinamide on bio-availability of Rifampicin when co-administered Rifampicin-Isoniazid-Pyrazinamide. TCNCYH. 36, 12-17.

Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of Rifampicin and Isoniazid (alone and their combination) in the rat. Int. J. Tuber. Lung Dis. 7, 797-803.

Matsushima, T., 2005. Miliary tuberculosis or disseminated tuberculosis. Intern. Med. 44, 687.

McIlleron, H., Wash, P., Burger, A., Folb, P., Smith, P., 2002. Widespread distribution of a single drug rifampicin formulation of inferior bioavailability in South Africa. Int. J. Tuber. Lung Dis. 6, 356-361.

Mehta, S.K., Kaur, G., Bhasin, K.K., 2009. Entrapment of Multiple Anti-Tb Drugs in Microemulsion System: Quantitative Analysis, Stability, and In Vitro Release Studies. J. Pharm. Sci. 99 (4) 1896-1911.

Melia, C.D., Washington, N., Wilson, C.G., 1994. Advantages and disadvantages of multiparticulate delivery systems. In: Melia, C.D., Washington, N., Wilson, C.G. (Eds.), Multiparticulate Oral Dosage Forms: Technology and Biopharmaceutics. Scottish Academic Press, Edinburgh, pp. 135–140.

Mitchison, D.A., 1985. The action of antituberculosis drugs in short-course chemotherapy. Tubercle. 66(3), 219–225.

Moes, A.J., 1993. Gastroretentive dosage forms. Crit. Rev. Ther. Drug Carr. Syst. 10 (2), 143–195.

Panchagnula, R., Agrawal, S., Kaul, C.L., 2001. Fixed-dose combinations in the treatment of tuberculosis. Indian J. Pharm. Sci. 63, 1-9.

Panchagnula, R., Agrawal, S., 2004. Biopharmaceutic and pharmacokinetic aspects of variable bioavailability of rifampicin. Int. J. Pharm. 271, 1–4.

Panchagnula, R., Kaur, K.J., Singh, I., Kaul, C.L., 1999. The WHO simplified study protocol in practice: investigation of combined formulations supplied by the WHO. Int. J. Tuber. Lung Dis. 3, S336– S342.

Rowe, R.C., 1985. Spheronization: A novel pill-making process. Pharm. Int. 6, 119-123.

Rubinstein, M. H., 2000. Tablets. In: Aulton, M. E. (Ed.), Pharmaceutics: The Science of Dosage Form Design. Churchill Livingstone, New York, pp. 305.

Sankar, R., Sharda, N., Singh, S., 2003. Behaviour of decomposition of Rifampicin in the presence of Isoniazid in the pH range 1-3. Drug Dev. Ind. Pharm. 29, 733-738.

Sassetti, C.M., Rubin, E.J., 2007. The open book of infectious diseases. Nat. Med.13, 3. http://naturemedicine.com/naturemedicine.

Savale, S.S., 2003. Dissolution study and its correlation with bioavailability of the drugs. Ph.D. Thesis, Gujarat University, Ahmedabad.

Schluger, N.W., 2005. The pathogenesis of tuberculosis. The first one hundred (and twenty-three) years. Am. J. Respir. Cell Mol. Biol. 32, 251–256.

Sherrington, P.J., Oliver, R., 1981. Compaction and other granulation methods. In: Goldberg, A.S. (Ed.), Granulation. Heyden, London, pp. 141-152.

Shishoo, C.J., Shah, S.A., Rathod, I. S., Savale, S. S., Kotecha, J. S., Shah, P.B., 1999. Stability of rifampicin in dissolution medium in presence of Isoniazid. Int. J. Pharm. 190, 109–123.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., 2001. Impaired Bioavailability of Rifampicin from its Fixed Dose Combination (FDC) Formulations with Isoniazid, Indian J. Pharm. Sci., 63(6), 443-449.

Singh, B.N., Kim, K.H., 2000. Floating drug delivery systems: an approach to oral controlled drug delivery *via* gastric retention, J. Control. Rel. 63 (3), 235– 259.

Singh, S., Bhutani, H., Mariappan, T.T., Kaur, H., Bajaj, M., Pakhale, S.P., 2002. Behaviour of uptake of moisture by drugs and excipients under accelerated conditions of temperature and humidity in the absence and the presence of light. 1. Pure anti-tuberculosis drugs and their combinations. Int. J. Pharm. 245, 37-44.

Singh, S., Bhutani, H., Mariappan, T.T., 2006. Quality problems of anti-tuberculosis fixed-dose combinations (FDCs): A way forward. Indian J. Tuberc. 53, 201-205.

Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.

Singh, S., Mariappan, T.T., Sharda, N., Kumar, S., Chakraborti, A.K., 2000a. The reason for an increase in decomposition of Rifampicin in the presence of Isoniazid under acid conditions. Pharm. Pharmacol. Commun. 6, 405- 410.

Singh, S., Mariappan, T.T., Sharda, N., Singh, B., 2000b. Degradation of Rifampicin, Isoniazid and Pyrazinamide from prepared mixtures and marketed single and combination products under acid conditions. Pharm. Pharmacol. Commun. 6, 491-494.

Singh, S., Mohan, B., 2003. A pilot stability study on anti-tuberculosis four drug fixed dose combination products. Int. J. Tuber. Lung Dis. 7, 298-303.

Smith, I., 2003. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin. Microbiol. Rev. 16, 463-496.

Sosnik, A., Carcaboso, A.M., Glisoni, R.J., Moretton, M.A., Chiappetta, D.A., 2009. New old challenges in tuberculosis: Potentially effective nanotechnologies in drug delivery. Adv. Drug Deliv. Rev. 62, 547-559.

Stithit, S., Chen, W., Price, J.C., 1998. Development and characterization of buoyant theophylline microspheres with near zero order release kinetics, J. Microencapsul. 15, 725–737.

Sutherland, I., 1976. Recent studies in the epidemiology of tuberculosis, based on the risk of being infected with tubercle bacilli. Adv. Tuberc. Res.19:1-63.

Wan, L.S.C., 1989. Manufacture of Core Pellet Formation and Growth. In: Ghebre-Sellassie, I., (Ed.), Pharmaceutical Pelletization Technology. Marcel Dekker, Inc., New York, pp.123–143.

WHO, 1999. Fixed dose combination tables for the treatment of tuberculosis. Report of an informal meeting held in Geneva.

WHO, 2009a. Tuberculosis. http://who.int/mediacentre/factsheets/fs104/en/index.html. Retrieved 12 November 2009. Fact sheet N104.

WHO, 2009b. Global Tuberculosis report. A short update to the 2009 report. WHO /HTM/TB/2009.426.

WHO, 2010. Treatment of tuberculosis: Guidelines for national programmes, 4th edition. WHO/HTM/TB/2009.420.

Whitehead, L., Fell, J.T., Collett, J.H., Sharma, H.L., Smith, A.M., 1998. Floating dosage forms: an in vivo study demonstrating prolonged gastric retention. J. Control. Rel. 55, 3–12.

Zak, A.F., Batuashvili, T.A., Shcedrin, V.I., 1981. Rifampicin preparations for internal use and their bioavailability. Antibiotiki. 26, 728-731.

Chapter 2

Development & Evaluation of Rifampicin formulation

"The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them"

.....William Lawrence Bragg

2

Development and evaluation of rifampicin formulation

2.1 Introduction

For the last forty years, rifampicin and isoniazid has been the mainstay of the tuberculosis therapy. Rifampicin is the only drug that has the unique capability to kill dormant tubercular bacilli (Ellard and Fourie, 1999; Shishoo *et.al*., 2001). Over the years, two serious problems have emerged with FDCs that includes (Laserson *et.al*., 2001; Shishoo *et.al.*, 2001; Immanuel *et.al*., 2003; Singh and Mohan, 2003; Bhutani *et.al*., 2004; Luyen *et.al*., 2005):

- The impaired and variable bioavailability of rifampicin from FDC formulations with isoniazid
- Poor stability of rifampicin containing FDCs

The use of substandard FDC ultimately results in the emergence of drug resistant TB and treatment failure (Panchagnula *et.al*., 1999; IUTALD/ WHO, 1999). It has now been proved, beyond doubt, that rifampicin interacts with isoniazid in the acidic medium of the stomach to form inactive isonicotinyl hydrazone (Singh *et.al*., 2000a; Singh *et.al*., 2000b; Shishoo *et.al*., 2001; Singh *et.al*., 2001; Sankar *et.al*., 2003). Shishoo *et.al*., in 2001 have shown that the decomposition of rifampicin interacts with isoniazid to the extent of 8.5 to 50% in the acidic environment of the stomach. This is reflected in the poor bioavailability from the anti TB - FDC formulation (Shishoo *et.al*., 2001). Hence, there is a critical need to redesign the currently available anti- TB FDCs.

Based on these observations, FDC product, devoid of both ‘bioavailability‘ and ‘stability’ problems, can be formulated by releasing rifampicin in the stomach and delivering isoniazid from the same formulation with a delay of 1-4 h.

Controlled release drug delivery systems are advantageous over conventional multidose delivery systems, particularly for long-term therapeutic effect and for the treatment of chronic diseases like tuberculosis. One of the essential factors for efficient therapeutic performance of the delivery system is the residence time of the drug at the absorption

site. The short residence time of oral controlled release dosage forms (CRDF) in the stomach leads to problems with bioavailability for certain classes of drugs. GRDDS are the formulations retained in the stomach for a prolonged period of time and release the drug in a controlled fashion. Unlike conventional CRDF, which release the drug in a controlled manner throughout the entire gastrointestinal tract, GRDDS retains in the stomach for an extended period of time and releases the drug in a controlled fashion (Moes, 1993; Singh and Kim, 2000; Whitehead *et.al*., 1998).

A prolonged residence time in the stomach is desirable for controlled release devices delivering drugs, which (i) are locally active in the stomach, e.g. misoprostol (Oth *et.al*., 1992), antacids (Fa´bregas *et.al*., 1994) and antibiotics against helicobacter pylori (Yang *et.al*., 1999), (ii) have an absorption window in the stomach or in the upper part of small intestine, e.g. L-dopa (Erni and Held, 1987), p-aminobenzoic acid (Ichikawa *et.al*., 1991a) (iii) are unstable in the intestinal or colonic environment, e.g. captopril or (iv) exhibit low solubility at high pH values, e.g. diazepam and chlordiazepoxide (Sheth and Tossounian, 1984). Furthermore, as the total gastrointestinal transit time of the dosage form is increased by prolonging the gastric residence time, these systems can also be used as extended release devices with a reduced 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 and magnetic systems.

2.2 Floating drug delivery system (FDDS)

These have 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). Few of the approaches that are used in designing intragastric floating systems are described below.

2.2.1 Hydrodynamically balanced systems (HBS)

These are single-unit dosage forms, containing one or more gel-forming hydrophilic polymers. Hydroxypropylmethylcellulose (HPMC) is the most common used excipient,

although hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), sodium carboxymethylcellulose (NaCMC), agar, carrageenans or alginic acid are also used (Reddy and Murthy, 2002; Hwang *et.al*., 1998). The polymer is mixed with drug and usually administered in a gelatin capsule. The capsule rapidly dissolves in the gastric fluid, and hydration and swelling of the surface polymers produces a floating mass. Drug release is controlled by the formation of a hydrated boundary at the surface. Continuous erosion of the surface allows water penetration to the inner layers, maintaining surface hydration and buoyancy (Reddy and Murthy, 2002). Madopar LP, based on this system, was marketed market by Roche during the 1980s (Jansen and Meerwaldtt, 1990). The main drawback is the passivity of the operation. It depends on the air sealed in the dry mass centre following hydration of the gelatinous surface layer and hence the characteristics and amount of polymer used (Hwang *et.al*., 1998). Effective drug delivery depends on the balance of drug loading and the effect of polymer on its release profile.

2.2.2 Gas-generating systems

Floatability can also be achieved by generation of gas bubbles. Carbon dioxide (CO_2) can be generated *in situ* by incorporation of carbonates or bicarbonates, which react with acid—either the natural gastric acid or co-formulated as citric or tartaric acid. An alternative is to incorporate a matrix with entrapped of liquid, which forms a gas at body temperature (Michaels, 1974; Michaels, 1975; Ritschel *et.al*., 1991). This approach has been used for single and multiple unit systems. In single unit systems, such as capsules (Chen *et.al*., 1998) or tablets (Baumgartner *et.al*., 2000; Xu *et.al*., 2001), effervescent substances are incorporated in the hydrophilic polymer, and CO_2 bubbles are trapped in the swollen matrix. *In vitro*, the lag time before the unit floats is <1 min. and the buoyancy is prolonged for 8 to 10 h (Baumgartner *et.al*., 2000).

2.2.3 Raft-forming systems

Here, a gel-forming solution (e.g. sodium alginate solution containing carbonates or bicarbonates) swells and forms a viscous cohesive gel containing entrapped CO_2 bubbles on contact with gastric fluid. Because raft-forming systems produce a layer on the top of gastric fluids, they are often used for gastroesophageal reflux treatment as with Liquid Gaviscon (Bardonnet *et.al*., 2006).

2.2.4 Low-density systems

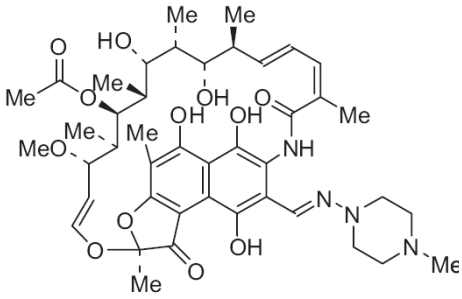
Gas-generating systems inevitably have a lag time before floating on the stomach contents, during which the dosage form may undergo premature evacuation through the pyloric sphincter. Low-density systems (<1 g/cm^3) with immediate buoyancy have therefore been developed. They are made of low-density materials, entrapping oil or air. Most are multiple unit systems, and are also called ‘‘microballoons’’ because of the low-density core (Sato *et.al*., 2004). At present, hollow microspheres are considered to be one of the most promising buoyant systems because they combine the advantages of multiple unit systems and good floating properties (Mitra, 1984). However, like all floating systems, their efficacy is dependent of the presence of enough liquid in the stomach, requiring frequent drinking of water.

Among the floating systems, multiple-unit formulations show several advantages over single unit drug delivery system: more predictable drug release kinetics, less chance of localised mucosal damage, insignificant impairing of performance due to failure of a few units, co-administration of units with different release profiles or containing incompatible substances, larger margin of safety against dosage form failure (Ghebre-Sellassie, 1989; Amighi *et.al*., 1998).

2.3 Drug Profile- Rifampicin

- Molecular formula $C_{43}H_{58}N_4O_{12}$

Chemical structure



- Generic name 5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl- 8-[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-b]furan-1,11(2H)-dione 21-acetate
- Molecular weight 822.94
- Solubility Freely soluble in chloroform and DMSO; soluble in ethyl acetate, methanol, tetrahydrofuran; slightly soluble in acetone,

	water, carbon tetrachloride
• Polarity (Log P)	3.719
• Acidity/basicity	pKa 1.7 for the 4-hydroxy and pKa 7.9 for the 3-piperazine nitrogen
• Stability	Very stable in DMSO; rather stable in water
• Melting point	183ºC
• Optimal human dosage	Dose 10 mg/kg, in a single daily administration, not to exceed 600 mg/day, oral or i.v
• *In vitro* potency	For *M. tuberculosis* H37Rv, MIC is 0.4 mg/ml (Rastogi, *et. al*., 1996).

Mechanism of action

Rifampicin inhibits the essential *rpoB* gene product b-subunit of DNA dependent RNA polymerase activity, acting early in transcription (Wehrli *et.al*., 1968). It is thought to bind to the ß-subunit, close to the RNA/DNA channel, and physically blocks the transit of the growing RNA chain after nucleotides have been added (Wehrli *et.al*., 1968; Engelburg-Kulka, *et.al*., 2004).

Spectrum of activity

Rifampicin is bactericidal with a very broad spectrum of activity against most gram-positive and some gram-negative organisms (including *Pseudomonas aeruginosa*) and *M. Tuberculosis*. Rifampicin has clinical efficacy against a wide variety of organisms, including *Staphylococcus aureus*, *Legionella pneumophila*, Group-A *Streptococcus*, *Brucella* spp., *Haemophilus influenzae*, and *Neisseria meningitidis*, as well as *in vitro* activity against penicillin-resistant *S. pneumoniae*, *N. gonorrhoeae*, *Chlamydia trachomatis*, *H. ducreyi*, and many gram-negative rods. Due to rapid emergence of resistant bacteria it is restricted to treatment of mycobacterial infections, where the customary use of combination drugs delays resistance development, and the treatment of asymptomatic meningococcal carriers (Petri, 2001).

Pharmacokinetics of Rifampicin

Absorption

Rifampicin is well absorbed from the gastrointestinal tract, with peak plasma levels achieved within 1 to 4 h after oral administration, although food may delay its absorption (Kebrele, 1970; Siegler, *et.al*., 1974).

After intravenous dose administration the plasma levels in adults are about 9 µg/ml (300 mg infusion) over 30 min. after infusion.

Distribution

Rifampicin readily diffuses into most organs, tissues, bones and body fluids, including exudates into tuberculosis infected lung cavities (Acocella, *et.al*., 1967). Therapeutic concentrations are achieved in saliva reaching 20% of serum concentrations. High concentrations appear in the lachrymal glands and tears. The urine is coloured orange to brick red. It is reported that rifampicin is highly protein bound to an extent of 84-91% (Jack, 1992). Tissue distribution occurs at a relatively fast rate. At physiological pH only about 25% of the drug is ionized while the molecule as a whole is lipid soluble. Levels of rifampicin in the cerebrospinal fluid are approximately one tenth of those achieved in the blood, although this may be increased in inflammatory states (Acocella, *et.al*., 1971; Nahata, *et.al*., 1990).

Metabolism

The principal pathways of metabolism of rifampicin involve desacetylation and hydrolysis. Desacetylation at the C-25 position results in a more polar and equally active compound, 25- desacetyl rifampicin (DAR), with increased capacity for biliary excretion. Depending on the dose of rifampicin, one-third to one-eighth may be excreted in the bile, either as a 25-DAR or as unchanged rifampicin. The unchanged rifampicin is reabsorbed, creating an enterohepatic circulation, whereas the 25-DAR is poorly absorbed (Teuinssen, *et.al*., 1984). The half-life of rifampicin is 3-5 h. Rifampicin, stimulates its own metabolism in liver and the biliary excretion of desacetyl rifampicin (Douglas and Macleods, 1999). On first dose administration on an empty stomach of 300 mg rifampicin, the serum concentration curves are similar to those following intravenous dosing, indicating little presystemic metabolism, but repeated administration induces hepatic endoplasmic reticular enzymes (Keberle, 1970). Rifampicin induces certain cytochrome P450s, mainly 3A4 isozyme. The bioavailability of the active, orally administered rifampicin decreased from 93% after the first single oral dose to 68% after 3 weeks of oral and intravenous rifampicin therapy. This is attributed to both, an increased hepatic metabolism and an induction of a prehepatic “first-pass” effect resulted from multiple rifampicin doses (Loos, *et.al*., 1985).

Excretion

Rifampicin is mainly eliminated in bile, gets reabsorbed and undergoes enterohepatic circulation. Amount excreted in urine increases with increasing doses and upto 30% of dose of 900 mg may be excreted in urine, about half of it within 24 h (Jack, 1992; Reynolds, 1993; Acocella, 1978). About 40% is excreted in bile. About 60-65% dose appears in feces. Within 24 h, 3-30% of unchanged drug and active metabolite get excreted in urine (600 mg single dose oral administration). 6-15% of dose is excreted in urine and 15% of dose appears as active metabolite (25-DAR) in urine. 7% of dose is excreted as inactive 3-FRSV (Acocella, 1978).

Drug-Drug interactions

Rifampicin induces certain cytochrome P450s, mainly 3A4 isozyme. The rifampicin dose of 600 mg/day was established partly to limit the CYP3A induction potential (Burman et al. 2001). The drug affects the metabolism of the following drugs: acetaminophen, astemizole, carbamazepine, corticosteroids, cyclosporin, dapsone, ketoconazole, methadone, phenobarbital, phenytoin, quinidine, terfenadine, theophylline, verapamil and warfarin (Douglas and McLeod, 1999).

Adverse effects of Rifampicin

Human adverse reactions: Hepatitis and serious hypersensitivity reactions including thrombocytopenia, hemolytic anaemia, renal failure have been reported. Asymptomatic elevations of serum transaminase enzymes, increase in serum bile acids and bilirubin concentrations can occur. Marked elevation of serum alkaline, phosphatase and bilirubin suggests rifampicin liver toxicity.

Cardiovascular: Hypotension and shock.

Respiratory: Shortness of breath.

CNS: Rare cases of organic brain syndrome have been reported (i.e. confusion, lethargy, ataxia, dizziness and blurring of vision).

Gastrointestinal: Nausea, vomiting, diarrhoea. Rifampicin causes orange-red staining of all body fluids (Sensi and Gressi, 1996; Petri, 2001).

Indications

The primary indications for rifampicin are for treatment of tuberculosis (pulmonary and extrapulmonary lesions) and for leprosy. It has recently been used for brucellosis (Petri, 2001).

Contraindications

Rifampicin is contraindicated in known cases of hypersensitivity to the drug. It may be contraindicated in pregnancy (because of teratogenicity noted in animal studies and since the effects of drugs on foetus have not been established) except in the presence of a disease such as severe tuberculosis. It is contraindicated in alcoholics with severely impaired liver function and with jaundice (Petri, 2001).

2.4 Formulation design

In the current study, in order to avoid interaction between rifampicin and isoniazid, both in formulation as well as *in-vivo*, a novel rifampicin and isoniazid FDC formulation is proposed, wherein, rifampicin and isoniazid will be released in different regions of the GIT, in order to avoid the interaction between them in the acidic environment of stomach.

The 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

Thus, the FDC design had modulated release of rifampicin so as to target it to the stomach *via*, gastroretention approach, floating drug delivery systems. A part of rifampicin dose was formulated as a loading dose in the form of multiparticulate system. Loading dose of rifampicin will be released immediately in stomach followed by its sustained release *via* gastroretentive floating formulation. The sustained release of rifampicin in stomach will help to maintain the concentration of rifampicin within therapeutic window, at its absorption maxima site for a prolonged period of time (Mariappan and Singh, 2003). Sustained release delivery system holds promise for reducing the dosing frequency and improving patient compliance, in the management of tuberculosis.

In the current chapter, formulation development of rifampicin will be discussed. The current chapter is subdivided in two parts, wherein,

Part A: Formulation development and evaluation of immediate release pellets of rifampicin (*loading dose*), will discussed.

Part B: Formulation development and evaluation of gastroretentive floating pellets of rifampicin (*maintenance dose*), will discussed.

2A

FORMULATION DEVELOPMENT AND EVALUATION OF IMMEDIATE RELEASE RIFAMPICIN PELLETS (*loading dose*)

2.5 Materials

Rifampicin was obtained as a gift sample from Cadila Pharmaceuticals Limited, Ahmedabad. The excipients and chemicals used in the formulation development and its evaluation like Crosspovidone, Ac-di-sol, Indion 414, Avicel PH101, Polyvinyl pyrolidone (PVP), Lactose, Chloroform etc are enlisted in Annexure1.

2.6 Methods

2.6.1 Preliminary screening of the excipients

Differential Scanning Calorimeter (DSC) was employed as a means to investigate the physicochemical compatibility between rifampicin and a number of commonly used excipients. Thermograms of several excipients with/without drug were obtained using a Differential scanning calorimeter, Perkin-Elmer-7 (Perkin Elmer, USA) instrument equipped with an intracooler. Indium and Zinc standards were used for the calibration of DSC. Weighed amount of powder samples were sealed in aluminum pans and heated at constant rate of 10°C/min. over the temperature range 25–350°C. The system was purged with nitrogen gas at the rate of 40 ml/min. to maintain inert atmosphere.

Compatibility of rifampicin was studied with, extrusion aid-microcrystalline cellulose (MCC), lactose; disintegrants and super disintegrants like sodium starch glycolate (SSG), Ac-Di-Sol, Cross povidone, Indion 414, binder- polyvinyl pyrrolidone (PVP K-90).

2.6.2 Material characterization

2.6.2.1 Loss on drying (LOD)

LOD was determined as per USP method (USP, 2007a). Rifampicin was heated at 60ºC for 4 h. Three parallel determinations were performed in each case.

2.6.2.2 Bulk and tapped density

The bulk density was determined by pouring weighed amount of materials into a graduated glass cylinder. The bulk density was calculated by dividing the weight by the occupied volume. The tapped density was determined using a tapped density tester (Lab Hosp, India) in which the glass cylinder was tapped 500 taps followed by 750 taps, if required (USP, 2007b). The tapped density was calculated in the same way as the bulk density. All measurements were carried out in triplicate.

2.7 Method of preparation of rifampicin pellets

2.7.1 Granulation

Rifampicin was blended with Avicel PH 101, Indion 414. The batch size was 250g of dry material and the rifampicin load varied from 50 to 85% (w/w). The blend was dry mixed for 5 min. at 60 rpm in a planetary mixer (Kalweka, Karnavati Eng. Ltd., India). The mixture was wetted with purified water (40 - 43% of the total mass) and PVP 90 solution and granulated for 5 min. using the same equipment and mixing speed.

2.7.2 Extrusion

The wet mass was extruded at an extrusion speed of 150 rpm by means of a gravity fed extruder (R.R Enterprise, Mumbai).

2.7.3 Spheronization

The extrudates were spheronized in a spheronizer (R. R. Enterprise, Mumbai) using a friction plate with cross-hatched geometry.

2.7.4 Drying

The pellets were dried in a fluidised bed dryer (Niro Aeromatic, Switzerland) at 50°C for 10 min.

2.7.5 Experimental design

A 3^2 Full Factorial Design (FFD) was used for the optimization of immediate release rifampicin formulation. Amount of superdisintegrant (Indion 414, X_1, %) and amount of soluble extrusion aid (Lactose, X_2, %) were the two factors (independent variables) studied. The responses (dependent variables) studied were porosity (Y_1, %), friability (Y_2, %) and amount of rifampicin released in 45 min. (Y_3, %). Table 2 summarizes

independent and dependent variables along with their levels. Various formulations were prepared as per the compositions mentioned in Table 3.

Table 2. Factors (independent variables), factor levels and responses (dependent variables) used in 3^2 full factorial experimental design

Factors	Factor level			Response
	-1	0	+1	Y_1= Porosity (%)
X_1=Amount of Indion (%)	0	4.0	8.0	Y_2= Friability (% w/w)
X_2= Spheronization speed (rpm)	600	800	1000	Y_3= Amount of rifampicin released in 45 min. (%)

Table 3. Immediate release rifampicin formulation compositions as per 3^2 full factorial experimental design

Formulation	Amount of Indion (%)	Spheronization speed
1	0.00	1000
2	8.00	800
3	8.00	600
4	0.00	800
5	0.00	600
6	8.00	1000
7	4.00	1000
8	4.00	600
9	4.00	800

2.8 Statistical analysis of the data and validation of the model

Various response surface methodology (RSM) computations for the current study were performed employing Design-Expert software® (Version 7.1.2, Stat-Ease Inc., Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. Statistical validity of the polynomials was established on the basis of Analysis of variance (ANOVA and the 3D response graphs were constructed using Design-Expert software. To validate the chosen experimental design and polynomial equations, optimum test condition was selected. The tests corresponding to this optimum condition and three additional random conditions were carried out in the experimental matrix to determine the validity of the model generated. Subsequently, the resultant experimental

data of the response properties were quantitatively compared with those of the predicted values. Also, the linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel.

2.9 Characterization of rifampicin pellets

2.9.1 Particle size distribution

Pellet size distribution (span) was carried out by Malvern Mastersizer (Malvern 2000, Malvern Instruments, UK). All the measurements were carried out in triplicate. Span was used as an indicator of particle size distribution. The 50^{th} percentile diameter of the cumulative particle size distribution was considered as mean pellet size (Koo and Heng, 2001).

2.9.2 Usable yield (% theoretical)

The usable yield of the pellets was determined from sieve analysis, which was carried out using a sieve shaker (EMS-8, Electrolab, India) equipped with (600-2360 µm) sieves, at amplitude of 2 mm, for 5 min. The pellet yield was calculated based on the pellet fraction between #14/22 and presented as the percent of the total pellet weight (Howard, *et.al*, 2006). This size fraction was used for all further measurements.

2.9.3 Pellet sphericity and shape analysis

The pellet spherecity and shape of the pellets were determined using an image analysis system. Photomicrographs of pellets were taken with a digital camera linked with a stereomicroscope system a stereomicroscope Leica S4E (Germany). The captured images were analysed by image analysis software (AnalySIS, v. 5.2, Soft Imaging System, Münster, Germany). Around 50 pellets were analysed for every batch. Each individual pellet was characterised by pellips (as described by Podczeck, *et.al*., 1999; Koo and Heng, 2001; Almeida-Prieto *et.al.*, 2007)

$$Pellips = \frac{P}{\pi \, x \, d_{max}} \qquad \text{--(1)}$$

Where, P is the perimeter and d_{max} is maximum diameter of the pellet, calculated directly by using Image analysis software.

2.9.4 Friability

The friability of the pellets (#14/ 22 fraction) was determined in Roche friabilator (EF-2, Electrolab, India). Weighed amount of pellets were subjected to friability test along with 24 steel balls (diameter about 2 mm) in Roche friabilator for 100 revolutions at 25 rpm and then sieved through a #22 sieve. The percent weight loss was then calculated (Howard *et.al*., 2006). Each batch was analysed in triplicate.

2.9.5 Mechanical crushing force

At least 20 pellets from the modal size fraction of each formulation were evaluated for their diametral crushing force using a tablet strength tester (EH 01, Electrolab, India) (Sousa *et.al*., 2002; Newton *et.al*., 2007).

2.9.6 Densities and angle of repose

The bulk density was determined by pouring weighed amount of pellets into a graduated glass cylinder. The bulk density was calculated by dividing the weight by the occupied volume. The tapped density was determined using a tapped density tester in which the glass cylinder was tapped 1000 times (750 taps followed by 250 taps) (USP, 2007a). All measurements were carried out in triplicate. Angle of repose was determined using reposograph.

2.9.7 Porosity

Pellet porosity was calculated using the following equation (Eq. 2), for percent effective porosity (Chopra *et.al*., 2001; Steckel and Mindermann-Nogly, 2004)

$$\%\varepsilon = [(\rho t - \rho b) / \rho b] \times 100 \qquad \text{--(2)}$$

Where ε = effective porosity, ρt = true density and ρb = bulk density. The true density of the powder formulation was determined in triplicate using Helium pycnometry (Smart Pycno 30, Smart Instruments, Mumbai).

2.9.8 Moisture content

The residual water content present in the pellets after drying was determined by using Karl Fischer titrator (Systronics Universal titrator 353, India), USP method I. The equipment was pre-calibrated and standardised with di-sodium tartrate. Pellets, approximately 250mg, were accurately weighed and immediately placed in the moisture

analyser for titration with Karl Fischer reagent. Each batch was analysed in triplicate (USP, 2007b).

2.9.9 Surface characterization

Morphological examination of the surface of pellets was carried out using a scanning electron microscope (SEM). SEM of pellets was obtained using JEOL JSM 6100 (JEOL, Japan). The particles were vacuum dried, coated with thin gold-palladium layer by sputter coater unit (JEOL, JFM 1100, Japan) and observed microscopically at an accelerating voltage of 5.0 kV.

2.9.10 Drug content

Rifampicin pellets were assayed by a validated dual wavelength spectrophotometric method using UV-Vis spectrophotometer (Shimadzu UV-2450, Japan) (Shishoo *et.al*., 1999; Savale, 2003). This method enables simultaneous quantification of the degradation product of rifampicin i.e., 3-FRSV. Wavelengths used were: 475nm and 507nm for rifampicin and 457 nm and 492nm for 3-FRSV.

2.9.11 Drug release study

In- vitro drug release studies of rifampicin pellets were carried out as per USP 30/NF 25 in USP apparatus I (SR8 Plus Hanson Research Corporation, Chatsworth, CA) (USP, 2007b). Rifampicin pellets equivalent to 300 mg were used for carrying out the dissolution studies. The test was carried out in USP dissolution test USP test apparatus-I, 100 rpm, 900 ml 0.1 N HCl. A sample of 5 ml was withdrawn and replaced with an equal amount of sample at 15 min., 30 min., 45 min., 60 min., 75 min., 90 min., 105 min. and at 180 min. The dissolution samples were analysed by dual wavelength spectrophotometric method for quantifying rifampicin and its degradation product 3-FRSV (Shishoo *et.al*., 1999).

2.10 Stability of Immediate release rifampicin pellets

The optimised rifampicin immediate release pellets prepared were subjected to stability studies. For this part of the study, the pellets were filled into empty hard gelatine capsule shells and were stored in tightly closed high density polyethylene (HDPE) containers and aluminium pack. The stability studies were carried out at room temperature and accelerated relative humidity conditions as per International conference on harmonization (ICH) guideline Q1A(R2) (ICH, 2003). The accelerated relative stability

conditions were maintained in a humidity chamber (EIE Instruments Pvt. Ltd., Ahmedabad) at 40°C ± 2°C/75% RH ± 5% RH. The stability samples were analysed at 1, 2, 3 and 6 M. The assay, water content and dissolution studies of these pellets were carried as per the methods described earlier. To ensure the equivalence in release profile of the stability samples with that of initial samples, the fit factor, similarity factor (f_2), was calculated. The formula used for calculating f_2 values is shown in Eq. (2):

$$f_2 = 50 \log\{[1+ 1/n\sum_{t=1}^{n}(R_t-T_t)]^{0.5} \text{ X } 100\} \quad \text{--(3)}$$

2.11 Results and Discussion

2.11.1 Preliminary experiments

Compatibility of rifampicin was studied with, extrusion aid-MCC, lactose; disintegrants and super disintegrants like SSG, Ac-Di-Sol, Cross povidone, Indion 414, binder- PVP, HPMC, Na CMC was studied using DSC. Rifampicin was found to be compatible with all the excipients.

Also, rifampicin was characterized for LOD, Bulk density and tapped density. The LOD of rifampicin was found to be less than 1% w/w (USP limits: NMT 2% w/w; USP, 2007a), bulk density was found to be around 0.71 gm/cm^3, while tapped density was found to be 0.62 gm/cm^3. These specifications were kept uniform throughout the rifampicin formulation development.

2.11.1.1 Selection of formulation variables

The goal of the preliminary experiments was to produce pellets with maximum yield and acceptable sphericity as visually observed. Avicel PH 101(extrusion- aid) had a favourable extrusion/spheronization behaviour, as it could be extruded with minimal resistance (generating limited friction and heat), the extrudate fragmented evenly during spheronization process and the fragments could be easily spheronized. However, when using microcrystalline cellulose as the only powder component and water as granulation liquid, a large amount of fines were generated. Addition of a binder was therefore required to obtain pellets with an acceptable pellet size distribution. The use of binder, PVP, improved the binding efficiency of the extrudates, yielding sufficiently large pellets after spheronization. However, use of higher grades of PVP like, PVP K-30/ K-90 yielded sticky extrudates, which promoted pellet agglomeration during

spheronization. A low viscosity PVP grade, Kollidon 90 was thus selected for further experiments, because it provided the best binding properties combined with minimal sticking of the extrudates during spheronization.

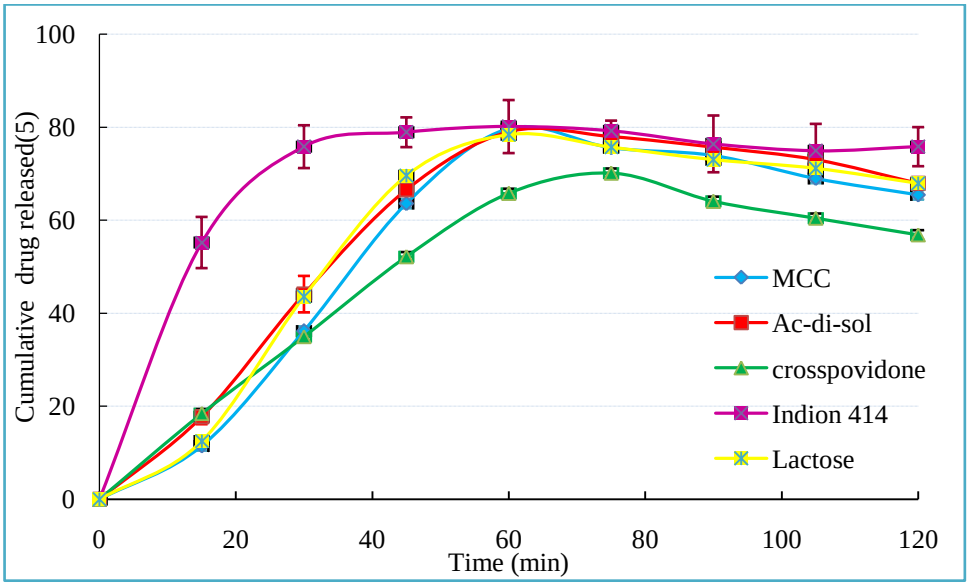
Pellet formulations prepared containing rifampicin (55% w/w dry mass), microcrystalline cellulose (43.5% w/w dry mass) and PVP (1.5% w/w dry mass) showed acceptable micromeritic properties, however, these pellets showed incomplete release of rifampicin (U.S.P. limit- NLT 75% (Q) in 45 min.) (USP, 2007a). This can be ascribed to the fact that for poorly water soluble drug are slowly dissolved from MCC pellets prepared by extrusion/spheronization. This slow dissolution rate is derived from the pronounced contraction of the pellet during the drying phase, that leads to reduced porosity. This in turn hinders the ingress of the dissolution medium into the pellet (Souto *et.al*., 2005). Hence, a part of Avicel 101 was replaced with lactose, a water soluble extrusion aid. This resulted in improvement of dissolution profile of the rifampicin but there was a significant increase in the size of beads and decrease in sphericity (Pellips< 1.00). A pellet with pellips equal to 1.0 is considered spherical and good for pharmaceutical processing (Hellén and Yliruusi, 1993). The observed difference could be ascribed to the ability of lactose to absorb more water, thereby, increasing the pellet size. On the other hand, the pellets containing higher amount of MCC tend to shrink after the removal of water due to a phenomena known as “crystallite-gel” (Kleinebudde, 1994; Paterakis *et.al*., 2002).

Consequently, it was decided to incorporate superdisintegrants into the pellet formulation. Crosscarmellose sodium, crosspovidone and Indion 414 were incorporated in the formulation and evaluated for the improvement in the dissolution profile of the rifampicin pellets. The drug release studies were carried out for the pellets containing different superdisintegrants, Crosscarmellose sodium, crosspovidone and Indion 414. A comparative release profile of rifampicin pellets using different superdisintegrants is shown in Fig 6. It can be seen from the graph that pellets prepared with a combination of lactose and superdisintegrants resulted in the improvement of dissolution profile of rifampicin. Among the three superdisintegrants, crosscarmellose sodium and crosspovidone were found to be less efficient in facilitating drug release from the rifampicin pellets even at a high level of 10% w/w and 5% w/w, respectively.

In general, the disintegrants promote absorption of moisture by promoting capillary action and swell, resulting in release of the drug. However, superdisintegrants loose this property on incorporation in pellet formulation. This may be due to the fact that water added during formulation process is absorbed by the disintegrant. This absorbed water causes the partial swelling of disintegrants during pelletization process. As a result, superdisintegrants cannot act as a swelling agent to push the dissolution of drug from the formulation during the dissolution test (Wlosnewski *et.al*., 2009).

However, in contrast to crosscarmellose sodium and crosspovidone, Indion 414 retained its functionality even after wet extrusion under high pressure and promoted dissolution of rifampicin (Fig 6). Therefore, amount of Indion 414 was selected as one of the variable in the optimization of rifampicin pellets and is discussed in detail in chapter later (section 2.11.2).

Fig 6. Comparative release profile of rifampicin pellets using different superdisintegrants (10% w/w) (n=6 ±SEM)



2.11.1.2 Selection of process variables

Formulations containing rifampicin (55% w/w, dry mass) drug, microcrystalline cellulose (13.5% w/w, dry mass), lactose (30%/w, dry mass) and PVP (Kollidon 90, 1.5% w/w, dry mass) were used to evaluate the influence of the following process variables: extrusion speed, spheronization speed, spheronization time and spheronization load.

The extrusion speed was kept constant at 150 rpm, throughout the trials, due to instrumental constraints. Also, various reports suggest that pellet quality is not affected by extrusion speed (Vervaet and Remon, 1996).

Using several spheronization speeds during preliminary tests revealed its major influence on pellet yield and sphericity: using a lower spheronization speed led to dumb-bell formation due to insufficient spheronization, while a higher spheronization speed promoted formation of spherical pellets. However, in that case the pellet yield was low due to excessive breaking of the extrudates. This process variable was therefore selected for further evaluation and is discussed in detail later in the section 2.11.2.

Preliminary experiments also showed that a spheronization time (around 10 min.) was sufficient to produce pellets with maximum yield and acceptable sphericity. Pellet spherecity was not improved on spheronizing pellets for longer duration, but promoted broadening of pellet size distribution and pellet agglomeration. The spheronization time was therefore fixed at 10 min. for further experiments.

A reduction in the sphericity of pellets was observed when using a higher spheronization load. This might be due to pellet agglomeration in case of a higher spheronization load. Newton *et.al*. (1995) studied the influence of spheronization load on the sphericity of MCC-based pellets and concluded that a longer spheronization time was needed to obtain spherical pellets in case of a higher spheronization load. However, for the economy reasons, a spheronization load of 250 g with 10 min. of spheronization duration, was used for further experiments, even though higher loads are used for commercial applications.

2.11.2 Optimisation of immediate release rifampicin pellets

A 3^2 FFD was used for the optimization of immediate release rifampicin formulation. Various formulations were prepared as per the compositions mentioned in Table 3. A mathematical relationship was generated between the factors (dependent variables) and responses (independent variables) using the statistical package Design-Expert for determining the levels of factors, which yield optimum dissolution responses. A second order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 \quad \text{--(4)}$$

Where b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; b_1 to b_5 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the coded levels of factors. The terms X_1X_2 and X_{2i} (i = 1 and 2) represent the interaction and quadratic terms, respectively.

The equation represents the quantitative effect of factors (X_1 and X_2) upon the responses (Y_1 and Y_2). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors. ANOVA was applied for estimating the significance of the model, at 5% significance level. A model is considered significant if the p-value (significance probability value) is less than 0.05.

2.11.2.1 Porosity

The porosity of the pellets is an important characteristic, which might influence the drug release profile in different ways. Both the factors, amount of Indion and spheronization speed were found to have significant influence on porosity of the immediate release rifampicin pellets. The results of ANOVA analysis of porosity, modelled as per experimental design is presented in Table 4.

Table 4. ANOVA results for porosity

Porosity	
Source	*p*-value
Model	**0.0027**
X_1	**0.0220**
X_2	**0.0115**
X_1^2	**0.0476**
X_2^2	0.4670
$X_1 X_2$	0.0679
R^2	0.9585
Adjusted R^2	0.8892

Significant terms are shown in bold type

Significant quadratic model ($p<0.05$), insignificant lack-of-fit test ($p>0.05$) and agreement of predicted and adjusted R-squared values allow data modelling (Table 5). Influence of amount of Indion and spheronization speed can be explained by a mathematical relationship Eq. 5.

$$Y_1 = 33.60 + 3.61\ X_1 + 4.58\ X_2 + 4.63\ X_1^2 - 1.18\ X_2^2 + 2.82\ X_1X_2 \quad \text{--(5)}$$

A positive sign of all the coefficients of the factors i.e., amount of Indion and spheronization speed signifies that the porosity of the pellets increases if the level of these factors increases. The influence of amount of Indion 414 and spheronization speed on porosity is shown by a response surface plot Fig 7. It can be inferred from the graph and mathematical relationship (Fig 7 and Eq. 5), amount of Indion and spheronization speed affects the porosity in almost a positive and linear fashion. On increasing the amount of Indion 414 and spheronization speed, the porosity of pellet is increased. However, the effect of amount of Indion 414 is more pronounced on the porosity as compared to the effect of speed of spheronization. This is in confirmation with scanning electron photomicrographs of immediate release rifampicin pellets containing Indion 414, having a highly porous pellet structure (Fig 8).

Fig 7. Response surface plot showing the influence of amount of Indion 414 and spheronization speed on porosity

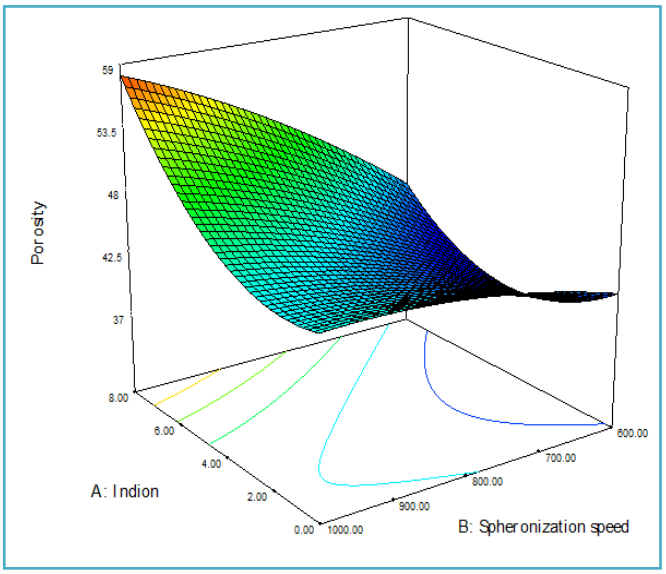
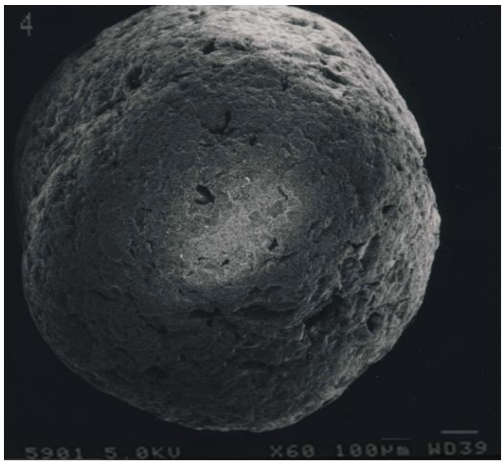


Fig 8. Scanning electron micrograph of immediate release rifampicin pellets containing superdisintegrant Indion 414



2.11.2.2 Friability

In general, friability indicates the ability of pellets to withstand the shear forces during handling and various pharmaceutical procedures. All the batches of rifampicin immediate release, Indion based pellets were found to have high mechanical strength, as indicated by their friability values (<0.1% w/w).

The results of ANOVA analysis of friability, modelled as per experimental design are presented in Table 5. ANOVA results suggest that linear model can be used for data fitting ($p<0.05$) and formulation optimization. Significant linear model ($p<0.05$), insignificant lack-of-fit test ($p>0.05$) and agreement of predicted and adjusted R-squared values allow data modelling (Table 5). The mathematical relationship that expresses the influence of amount of Indion 414 and spheronization speed on friability is given in Eq. 6.

$$Y_2 = 0.46 + 0.043\ X_1 + 0.0083\ X_2 \quad \text{--(6)}$$

ANOVA results in Table 5 reveals that none of the factors or their interaction product was found to have significant effect on friability of the pellets. Positive coefficients of both the factors indicate an increase in friability with the increase in the amount of Indion and spheronization speed. Response surface plot showing the influence of amount of Indion 414 and spheronization speed on friability is depicted in Fig 9. It can be seen that amount of Indion 414 and spheronization speed has a linear influence on the friability. However, the effect of amount of Indion 414 on friability is prominent in comparison to the effect of spheronization speed. This is apparent from the positive, smaller coefficient of spheronization speed in Eq. 6. Thus it can be concluded that with

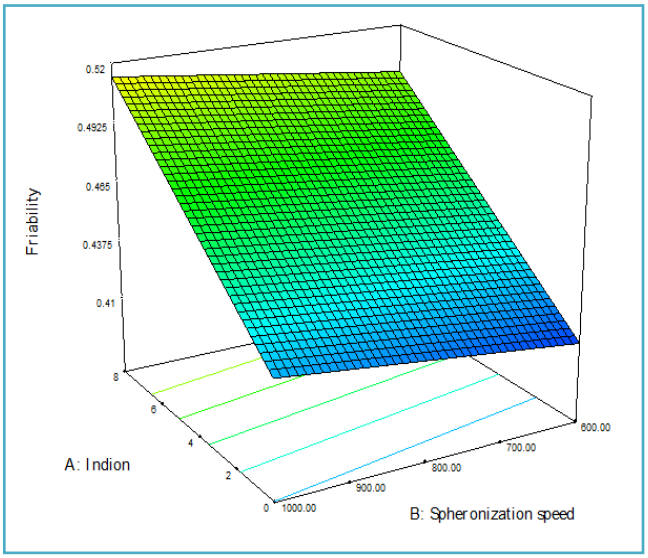
increase in amount of Indion 414, friability of rifampicin immediate release pellets increases. This might be due to the fact that, Indion 414 imparts a porous structure to the pellets (Fig 8), leading to a more friable structure of pellets. Hence, the amount of Indion 414 needs to be optimized in such a way that pellets have maximum porosity with minimum friability.

Table 5. ANOVA results for friability

Friability	
Source	*p* value
Model	**0.0426**
X_1	**0.0168**
X_2	**0.0451**
R^2	0.9910
Adjusted R^2	0.9297

Significant terms are shown in bold type

Fig 9. Response surface plot showing the influence of amount of Indion 414 and spheronization speed on friability



2.11.2.3 Pellet sphericity

One of the important objectives of pellet preparation (pelletization) is to produce spherical and smooth particles. Spherical particles help in the transfer of materials due to their good flow characteristics (Ghebre-Sellassie, 1989; Vertommen, *et.al*., 1997). Pellets provide a solid dosage form with several advantages. Their size and shape, particularly spherical, provide-

(a) Reproducible packing to allow high speed subdivision of bulk by volume,

(b) A free flowing system,

(c) A minimum surface area to volume ratio and no sharp corners, which allows the application of polymer coatings for controlled drug release (Chopra *et.al*., 2001).

In pharmaceutical literature, there are various shape factors that are used to describe pellets shape. Each shape factor has its threshold value that separates spherical pellets from non spherical pellets. In current study, the quality of the pellets was assessed using pellips as the indicators of pellet shape. A pellet with pellips equal to 1.0 is considered spherical and good for pharmaceutical processing (Hellén and Yliruusi, 1993).

The sphericity of pellets was determined by image analysis for all the batches. The results of ANOVA analysis of pellips modelled in the experimental design are presented in Table 6. Significant linear model ($p<0.05$), insignificant lack-of-fit test ($p>0.05$) and agreement of predicted and adjusted R-squared values allow data modelling. Spheronization speed, as well as its quadratic function were found to be significant factors ($p<0.05$), while the amount of Indion 414 level did not have a significant influence on pellet sphericity. The regression equation in terms of the coded factor values is presented in the Eq. 7.

$$Y_3 = 1.04 - 0.010\ X_1 + 0.0083\ X_2 - 0.017\ X_1^2 - 0.11\ X_2^2 - 0.00\ X_1X_2 \quad \text{--(7)}$$

Table 6. ANOVA results for Pellips

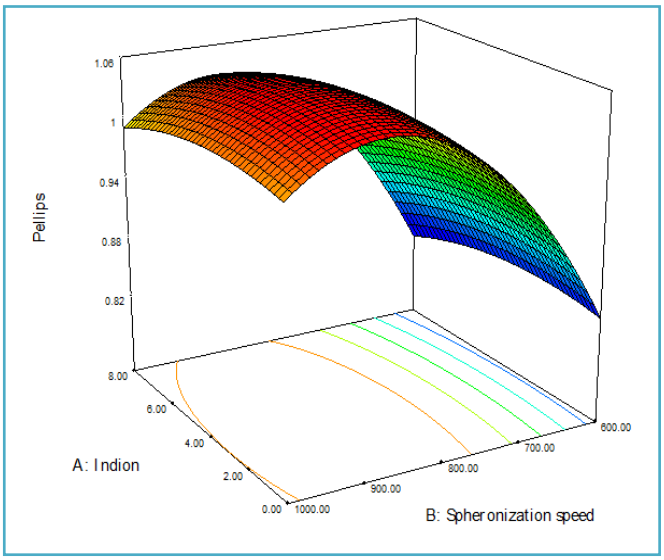
Pellips	
Source	*p*-value
Model	**0.0112**
X_1	0.3549
X_2	**0.0028**
X_1^2	0.3708
X_2^2	**0.0067**
$X_1\ X_2$	1.0000
R^2	0.9775
Adjusted R^2	0.9399

Significant terms are shown in bold type

A three-dimensional (3-D) response surface plot generated using the statistical model obtained from multiple regression analysis is presented in Fig 10 to observe the effect of changing independent variables on pellips of rifampicin pellets. The effect of

spheronization speed is evident from Eq. 7 and Fig 10. It can be concluded from Fig 10 that a region of maxima lies between, medium to higher speed of spheronization. Sphericity of pellets, as indicated by pellips, is achieved maximum, when spheronization speed is around 800-900 rpm (Fig 10).

Fig 10. Response surface plot showing the influence of amount of Indion 414 and spheronization speed on pellips



2.11.2.4 Drug release

In vitro drug release study was carried out in USP apparatus-I and amount of drug released at 45 min. was analysed by dual wavelength spectrophotometric method. All the formulation batches showed comparable drug release at 45 min. The results of ANOVA for the drug release modelled in experimental design suggest significant effect of the individual factors and their quadratic function on the response (Table 7). The relationship between amount of Indion 414 and spheronization speed is expressed in Eq. 8.

$$Y_3 = 71.91 + 8.61\ X_1 + 2.54\ X_2 - 4.90\ X_1^2 + 1.52\ X_2^2 + 0.33\ X_1X_2 \qquad \text{--(8)}$$

To observe the effect of changing independent variables on release of rifampicin from the pellet, a 3-D response surface plot was generated using the statistical model obtained from multiple regression analysis (Fig 11). Pronounced effect of amount of Indion, as seen in Fig 10, is apparent from the higher values of coefficients of individual factor (amount of Indion) and its quadratic factor in Eq. 8. A 'region of maxima' for maximum amount of drug release lies in between 6.00% to 8.00% w/w of Indion (Fig 11).

Table 7. ANOVA results for drug release at 45 min.

Drug release at 45 min.	
Source	*p* -value
Model	**0.0002**
X_1	**<0.0001**
X_2	**0.0015**
X_1^2	**0.0011**
X_2^2	**0.0306**
$X_1 X_2$	0.3216
R^2	0.9983
Adjusted R^2	0.9954

Significant terms are shown in bold type

A comparative release profile of rifampicin from pellets containing different amount of Indion 414 is shown in Fig 12. It was found that increasing the amount of Indion 414 from 2% to 6% w/w significantly improved the dissolution profile at 30 min. However, on further addition of Indion 414, significant improvement was not observed in the dissolution profile. Hence, it was concluded that Indion 414 is highly efficient disintegrating agent at 6% w/w, which resulted in significant improvement in drug release in dissolution medium without rendering pellets mechanically weaker (Fig 12).

Fig 11. Response surface plot showing the influence of amount of Indion 414 and spheronization speed on drug release

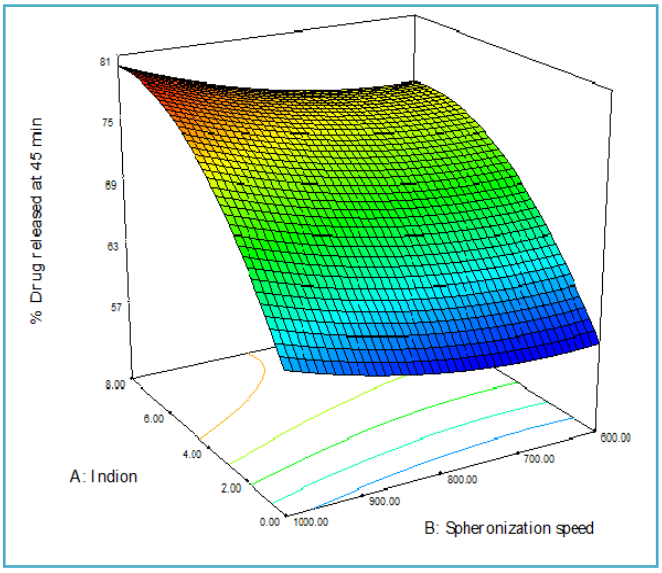
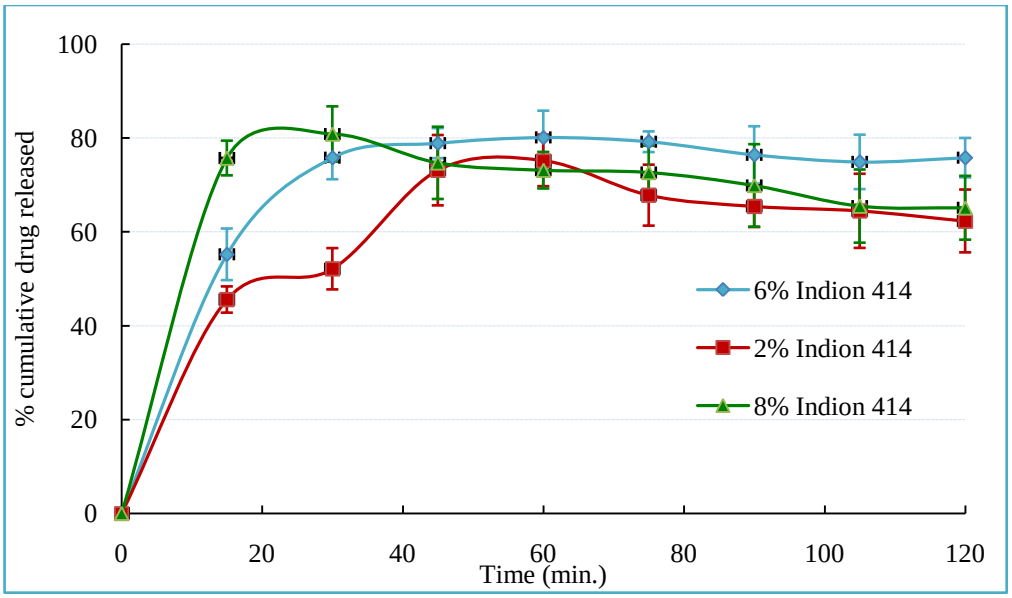


Fig 12. Release profile of rifampicin from pellets containing different levels of Indion 414 (n=6 ±SEM)



2.12 Validation of multiple response optimization model

In order to assess the reliability of the developed mathematical model, formulations corresponding to optimum composition and two additional random compositions covering the entire range of experimental domain were performed. For each of these formulations, the responses were estimated by the use of generated mathematical models and by the experimental procedures. The formulation parameters of the optimum and the random check points, their experimental and predicted values for all the four response variables are listed in Table 8. With the help of polynomial equation, the process was optimized for the responses. The final optimal experimental parameters were calculated by satisfying the requirements for each response in the set. Thus, to obtain immediate release rifampicin pellets it is desirable to have spherical pellets (Y_3, pellips=1.00) with maximum porosity (Y_1) and minimum friability (Y_2) along with maximum drug release at 45 min. (Y_4). For, optimization of process, constraints were applied and the optimal calculated parameters were

- Amount of Indion, X_1 = 6.00% w/w
- Spheronization speed, X_2 = 800 rpm

The above-mentioned optimized formulation was evaluated for all the parameters and showed low values of prediction percentage error indicating that the predicted and observed values are in good agreement (Table 8). Thus, the lower magnitudes of the error in current study indicate the robustness of the model used for the optimization of the rifampicin immediate release pellets. A summary of various micromeritic

characteristic and drug content of the optimized rifampicin immediate release pellets prepared with Indion 414 at 6% w/w (Table 9).

Table 8. The experimental and predicted values for all the eight responses (Y_1 to Y_4) along with percentage prediction error* observed for optimum formulation (A) and random formulation (B and C)

Formulation (X_1 %, X_2 rpm)	Response	Predicted Value	Experimental value	% PE*
A **6.00, 800** ***(Optimum)***	Y_1	50.5	50.00	-1.00
	Y_2	0.46	0.45	-2.23
	Y_3	0.98	0.99	1.01
	Y_4	79.5	81.2	2.09
B **8.00, 10000**	Y_1	48.1	46.5	-3.44
	Y_2	0.51	0.52	1.92
	Y_3	0.99	0.95	-4.21
	Y_4	80.02	78.5	-1.94
C **4.00, 600**	Y_1	43.5	42.5	-2.35
	Y_2	0.43	0.40	-7.50
	Y_3	0.88	0.90	2.22
	Y_4	72.1	73.4	1.77

*****Percent Prediction Error (PE) was calculated using the formula (Experimental Value – Predicted Value) / Experimental Value x 100.

Table 9. Summary of results of various micromeritic evaluation parameters and drug content of optimized rifampicin immediate release pellets prepared with Indion 414 at 6% w/w

Evaluation Parameter	Rifampicin pellets ± S.D
Diameter, d (0.9) (µm)	1444.716 ± 118.40
Roundness Score	1.0000±0.0413
Pellips	0.960±0.0437
Usable yield (%)	90.08
Friability	< 1%
Crushing Strength (N)	5.2 ± 0.87
Porosity (%)	46.14 ± 2.013
Water Content (%)	3.12 ± 1.09
Drug content (%)	95-105

2.13 Stability studies

The quality of a drug product changes with time under the influence of environmental factors such as temperature, humidity and light. The purpose of stability testing is to investigate those changes, to establish a shelf life for the drug product and to recommend storage conditions, which will be applicable to all future batches of the tested drug product manufactured and packaged under similar circumstances (Lusina *et.al*., 2005).

The amount of rifampicin and water content in the rifampicin pellets on stability is listed in Table 10. The accelerated stability data shows that rifampicin immediate release pellets are stable with drug content in the range of 98.99-100.67% and water content between 3.22- 4.55% w/w. It can be concluded that rifampicin content and water content of the rifampicin pellets do not changed significantly.

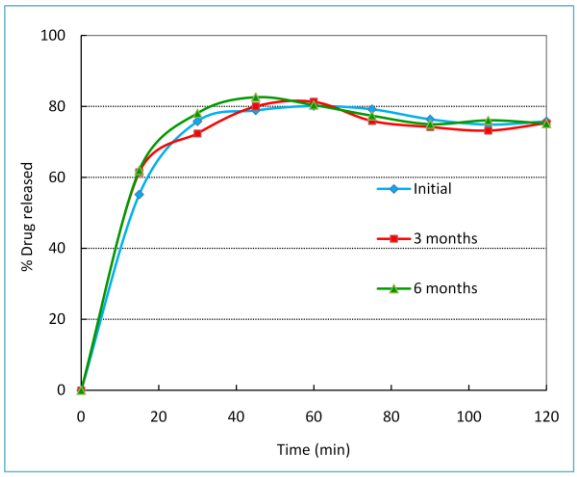
The release profile of rifampicin from the immediate release pellets when subjected to stability studies at 40°C ± 2°C/75% RH ± 5% RH and at room temperature are shown in Fig 13. It can be seen that significant change was not observed in the release profile during 6 M of storage at accelerated condition and at room temperature (Fig 13).

Table 10. Assay and water content of stability samples of rifampicin pellets

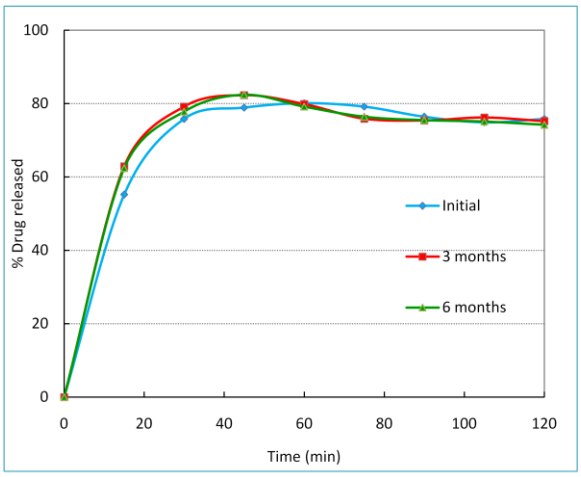
		40°C ± 2°C/75% RH ± 5% RH		At room temperature	
		HDPE	Aluminium strip	HDPE	Aluminium strip
Assay (%)	1M	100.45	99.75	99.76	100.15
	2M	99.53	100.02	100.23	98.99
	3M	100.67	100.23	99.78	99.98
	6M	100.43	99.69	100.54	100.11
Water Content (%w/w)	1M	4.11	3.22	3.87	3.88
	2M	4.47	3.35	4.08	3.99
	3M	3.65	4.55	4.15	4.18
	6M	3.88	4.28	4.29	3.35

However, to statistically establish the similarity between all the three release profiles, similarity factor, f_2 was applied. The f_2 similarity factor of rifampicin immediate release pellets after 6 M accelerated stability study and at room temperature, when compared with their initial values, was found to be > 90. Based on f_2 factor, it could be concluded that the pellets were stable at room temperature and also when subjected to accelerated stability studies.

Fig 13. Dissolution profile of initial, 3 M and 6 M stability sample of immediate release rifampicin pellet



a. Rifampicin immediate release pellets at 40 °C/75% RH



b. Rifampicin immediate release pellets at room temperature

Drug content with in pharmacopoeial limits, constant water content and values of similarity factor above 50 indicates that behaviour of rifampicin pellets are not affected by the accelerated stability conditions and are stable.

2.14 Conclusions

The immediate release rifampicin pellets were prepared using extrusion-spheronization and characterised. Based on various physico-mechanical properties and release characteristics, the following conclusion can be made:

- Immediate release rifampicin pellets were prepared using extrusion-spheronization. It was observed that use of microcrystalline cellulose (Avicel PH 101) facilitates the formation of spherical beads, however showed incomplete dissolution of rifampicin (U.S.P. limit- NLT 75% (Q) in 45 min.). It was found that replacing a part of MCC with lactose, a water soluble extrusion aid, and results in improvement of dissolution of rifampicin, however, there is a significant increase in the size of beads and decrease in sphericity.

  The observed difference could be ascribed to lactose ability to absorb more water increasing the pellet size, since the pellets containing higher amount of MCC tend to shrink after the removal of water, a phenomena known as "crystallite-gel".
- Superdisintegrants, Crosscarmellose Sodium and Crosspovidone were found to be less efficient in facilitating drug release from rifampicin pellets. While, Indion 414 retained its functionality even after wet extrusion under high pressure and efficiently increased the drug release from the pellets.
- Using response surface optimization, immediate release rifampicin pellets were prepared with satisfactory micromeritic parameters, mechanical and release profile. The amount of Indion 414 and spheronization speed has significant effect on the pellet physical and release characteristic. The optimum amount of Indion 414 and spheronization speed were found to be 6% w/w and 800 rpm respectively. The optimised rifampicin pellets were found to have: Usable yield > 90%, narrow pellet size distribution, % fines nil, roundness score and pellips near to 1, friability less than 1% and dissolution NLT 75% (Q)in 45 min.).
- The optimised rifampicin pellets were subjected to accelerated stability conditions (40°C ± 2°C/75% RH ± 5%) and at room temperature for 6 M. The rifampicin pellets were found to be stable (Assay 98.99-100.67%; water content 3.22-4.47% and dissolution NLT 75% (Q) in 45 min.).

B

FORMULATION DEVELOPMENT AND EVALUATION OF FDDS OF RIFAMPICIN *(maintenance dose)*

2.15 Materials

Rifampicin was obtained as a gift sample from Cadila Pharmaceuticals Limited, Ahmedabad. Table 12 lists the excipients used in the formulation. The grades and source of excipients and solvents like Avicel PH 101, HPMC K4M, Methocoel A15LV, Eudragit® NE 30D, Polyethylene Glycol (PEG) 6000, Sodium Carboxymethyl Cellulose (NaCMC), Sodium bicarbonate ($NaHCO_3$), Sodium Alginate, Lactose, Hydrochloric acid, Chloroform, Triethyl citrarte (TEC) used are mentioned in Annexure 1.

2.16 Methods

During preliminary experimentation, various approaches were adopted for preparing floating drug delivery system of rifampicin, which included:

- **Rifampicin floating pellets using extrusion -spheronization**
- **Rifampicin floating pellets based on effervescent technique**
- **Rifampicin floating tablet**

2.16.1 Approach I- Preparation of rifampicin floating pellets using extrusion-spheronization

Various trials with different polymers like HPMC K4M, Methocoel A15LV, NaCMC, Sodium bicarbonate, Sodium alginate etc were taken. Extrusion- Spheronization method was adopted for preparing floating rifampicin pellets.

2.16.1.1 Granulation

Rifampicin was blended with Avicel PH 101 and polymers like HPMC K4M/ Methocoel A15LV/ sodium carboxy methyl cellulose / sodium alginate. The batch size was 250 g with rifampicin 50% (w/w). The powders were dry mixed for 5 min. in a planetary mixer (Kalweka, Karnavati Eng. Ltd., India). The mixture was wetted with purified water (40 – 43% of the total mass) / hydroalcoholic solution and granulated for 5 min. using the same equipment.

2.16.1.2 Extrusion

The wet mass was extruded at an extrusion speed of 150 rpm by means of a gravity fed extruder (R.R Enterprise, Mumbai).

2.16.1.3 Spheronization

The extrudates were spheronized (at 800 rpm for 8 min.) in a spheronizer (R. R. Enterprise, Mumbai) using a friction plate with cross-hatched geometry.

2.16.1.4 Drying

The pellets were dried in a fluidised bed dryer (Niro Aeromatic, Switzerland) at 50°C for 10 min.

2.16.2 Approach II-Preparation of rifampicin multiple-unit FDDS based on effervescent technique

The spherical drug loaded core pellets were prepared by extrusion–spheronization process followed by coating the core pellets with effervescent component (like sodium bicarbonate) and HPMC (binder) and gas-entrapping polymeric membrane (Eudragit® NE 30D) (Sungthongjeen *et.al*., 2006).

2.16.2.1 Preparation of core rifampicin pellets

2.16.2.1.1 Granulation

Rifampicin was blended with Avicel PH 101. The batch size was 250 g of dry material. The powders were dry mixed for 5 min. in a planetary mixer (Kalweka, Karnavati Eng. Ltd., India).The mixture was granulated with Kollidon 90, binder solution.

2.16.2.1.2 Extrusion

The wet mass was extruded at an extrusion speed of 150 rpm by means of a gravity fed extruder (R.R Enterprise, Mumbai).

2.16.2.1.3 Spheronization

The extrudates were spheronized (at 800 rpm for 8 min.) in a spheronizer (R. R. Enterprise, Mumbai) using a friction plate with cross-hatched geometry.

2.16.2.1.4 Drying

The pellets were dried in a fluidised bed dryer (Nero Aeromatic, Switzerland) at 50°C for 10 min.

2.16.2.2 Coating of the core rifampicin pellets

The core pellets were coated with two successive layers: an effervescent substance, sodium bicarbonate, as an inner effervescent layer followed by an aqueous colloidal polymethacrylate, Eudragit® NE 30D, layer as an outer gas-entrapped polymeric membrane.

The effervescent agent, sodium bicarbonate, was incorporated into HPMC solution and plasticized with TEC followed by its coating onto the core pellets. The coating of effervescent layer was carried out till 10-12% weight gain. The coating solution was sprayed onto the core pellets in a fluid bed coater (Niroaeromatic, Switzerland). The conditions for layering are as follows:

preheating temperature, 40 °C; preheating time, 10 min.; inlet temperature, 40 °C; product temperature, 32-35 °C; spray nozzle diameter, 1.00 mm; atomizing air pressure, 1 bar; air flow rate 80m^3/h; spray rate 1.5 ml/min.; post drying at 40°C for 10 min. The $NaHCO_3$-layered pellets were dried in the fluidized bed coater for 30 min. at 50 °C to evaporate the residual moisture.

The $NaHCO_3$-layered rifampicin pellets were subsequently coated with an aqueous colloidal polymethacrylate dispersion (Eudragit® NE 30D) to achieve a weight gain of 10% (w/w) to obtain the complete multiple-unit FDDS. The coating conditions were as follows:

Load, 300 g; preheating temperature, 45 ◦C; preheating time, 10 min.; inlet temperature, 48-50ºC; outlet temperature, 38-40ºC; atomizing air pressure, 1bar; spray rate, 3–5 ml/min. The pellets were further dried in fluidized bed coater at 50ºC for 10 min., in order to evaporate the residual moisture in the polymeric coatings.

2.16.3 Approach III- Preparation of floating rifampicin tablet

The approach used for the preparation of floating tablets of rifampicin was based on Hydrodynamically Balanced System (HBS) in combination with a gas generation component. For HBS system, the polymer selected were hydroxypropyl methylcellulose

K4M, Carbopol 971P, while sodium bicarbonate will be used as a gas generating component. It is expected that the initial lag time to float required by the tablet would be reduced with the help of gas generating component of the formulation. Meanwhile, the polymer will swell and reduce the density of the formulation less than gastric content resulting in floating of the tablet.

2.16.3.1 Method of preparation

Rifampicin (150 mg) and microcrystalline cellulose (5% w/w) was granulated with purified water. A part of microcrystalline cellulose was added extra granularly. The wet coherent mass was passed through #22 sieve, and the granules were dried in oven at 60ºC for 15-20 min. Moisture content of the dried granules was measured on moisture balance (HB43-S, Mettler Toledo, Ohio, USA). A polymeric blend of HPMC K4M, Carbopol 971P was prepared as per the design and compositions mentioned in Table 11 and 12, respectively. Sodium bicarbonate (1%w/w) was sifted through #60 sieve and was geometrically mixed with the polymeric blend. Blend of polymers containing sodium bicarbonate and microcrystalline cellulose was then added extragranularly to the dried and sifted rifampicin granules. This was followed by the addition of magnesium stearate (1.5% w/w). Gastroretentive tablets of rifampicin with 200 mg average weight were prepared by compression on a single station tablet machine (Cadmach Machinery Co, Ltd, Ahmedabad, India). Each tablet contained 150 mg of rifampicin.

2.16.3.2 Experimental design

Optimization of rifampicin floating tablet was carried out using a 3^2 FFD. Amount of HPMC K4M (X_1,%) and amount of Carbopol (X_2,%) were the two factors (independent variables) studied. The responses (dependent variables) studied were floating lag time (Y_1, min.) and floating duration (Y_2, h) and $t_{80\%}$ (Y_3,%). Table 11 summarizes independent and dependent variables along with their levels. Various formulations were prepared as per the compositions mentioned in Table 12.

Table 11. Factors (independent variables), factor levels and responses (dependent variables) used in 3^2 full factorial experimental design

Factors	Factor level			Response
	-1	0	+1	Y_1= Floating lag time (min.)
X_1=Amount of HPMC K4M	5.0	10.0	15.0	Y_2= Floating duration (h)
X_2= Amount of Carbopol 971P	2.0	6.0	10.0	Y_3= $t_{80\%}$

Table 12. Floating rifampicin formulation compositions as per 3^2 full factorial experimental design

Formulation	Amount of HPMC K4M (%)	Amount of Carbopol 971P (%)
1	5.00	10.00
2	15.00	6.00
3	15.00	2.00
4	5.00	6.00
5	5.00	2.00
6	15.00	10.00
7	10.00	10.00
8	10.00	2.00
9	10.00	6.00

2.16.3.3 Evaluation of granules

The flowability of rifampicin granules was estimated by Carr's index, the Hausner Ratio, particle size distribution and the angle of repose. Rifampicin tablets were characterized on the basis of crushing strength (ET 101, Electrolab tablet tester, Electrolab India, Mumbai).

2.16.3.4 Evaluation of floating property of rifampicin formulation

2.16.3.4.1 *In vitro* floating duration

The floating duration is defined as the time period for which the tablet constantly floats on the surface of the medium. The *in vitro* floating behaviour of rifampicin formulation was carried out in USP apparatus II with 900 ml of 0.1 N HCl at 37ºC ± 0.05º at a stirring rate of 50 rpm (n=6). The floating duration of rifampicin formulation was determined by visual observation (Baumgartner, *et.al*., 2000).

2.16.3.4.2 *In vitro* floating lag time determination

This was determined in USP apparatus II with 900 ml of 0.1 N HCl at 37ºC ± 0.05º at a stirring rate of 50 rpm (n=6). The time interval upper one-third of the dissolution vessels was measured for each of the rifampicin formulation (Baumgartner, *et.al*., 2000).

2.16.3.4.3 *In vitro* release studies

In vitro release studies of rifampicin floating tablet was carried out in USP type I dissolution apparatus (Hanson research, Chatsworth, USA) 900 ml dissolution medium at 37±0.5 °C and the rotating speed was 100 rpm. The dissolution samples withdrawn at 15 min., 30 min., 60 min., 120 min., 180 min., 240 min., 300 min., 360 min., 480 min. and 520 min. were analysed by dual wavelength spectrophotometeric method for rifampicin and its degradation product 3-FRSV (Shishoo *et.al*., 1999).

2.16.3.5 Statistical analysis of the data and validation of the model

Various response surface methodology (RSM) computations for the current study were performed employing Design-Expert software (Version 7.1.2, Stat-Ease Inc., Minneapolis, USA). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. Statistical validity of the polynomials was established on the basis of ANOVA and the 3D response graphs were constructed using Design-Expert software. To validate the chosen experimental design and polynomial equations, optimum test condition was selected. The tests corresponding to this optimum dissolution condition and three additional random compositions were prepared in the experimental matrix to determine the validity of the model generated. Subsequently, the resultant experimental data of the response properties were quantitatively compared with those of the predicted values.

2.17 Assessment of *in vivo* gastroretention using Gamma-scintigraphic study

Gamma-scintigraphic studies were carried out to determine the location of rifampicin floating formulation on oral administration and the extent of its transit through the gastrointestinal tract. The study was performed at Gujarat Cancer Research Institute (GCRI), Ahmedabad. The study protocol was approved by the Institutional Ethics Committee of B. V. Patel PERD Centre. The study was conducted in accordance with the Declaration of Helsinki ethical principles (WMA, 2008).

Technitium-99m ($^{99m}TcO^{4-}$) is the radioisotope of choice for nuclear medicine imaging studies. It has a short half-life of 6.03 h and is easy and inexpensive to produce.

2.17.1 Subjects

Six healthy male and female volunteers participated in gamma scintigraphic studies. The ages of the volunteers ranged from 24 to 39 years. Their weights varied from 62 to 97 kg

and their body mass indices (BMI) from 19 to 25 kg/m^2.Only non-smokers were selected for the study. Subjects underwent a screening 14 days prior to the day of dosing and were judged healthy on the basis of medical history, physical examination, electrocardiogram and investigation of biochemical, immunological, parasitological and haematological parameters in blood and urine.

Each volunteer was informed about possible risks and adverse effects of taking the study formulations. Written informed consent to participation in the study was obtained. During the study a labelled formulation was administered only once to each study subject (single dose study).

2.17.2 Method of radiolabelling

^{99m}Tc was eluted as pertechnetate ($^{99m}TcO^{4-}$), with sodium chloride 0.9% from a molybdenum-99 generator. Radio labelling efficiency was evaluated with ITLC-SG strips as stationary phase and acetone (100%) as mobile phase.

$$\% \text{ Radiolabelling} = \frac{\text{Radioactivity (counts) retained in the lower half of the strip}}{\text{Initial radioactivity associated (total counts present) with the strip}} \times 100 \qquad \text{---(9)}$$

The rifampicin floating tablets were radiolabelled with 500 microcurie of $^{99m}TcO^{4-}$ (18.5 MBq). After incorporating ^{99m}Tc onto rifampicin tablet, ^{99m}Tc activity in the dosage form was assessed using dose calibrator. Marketed rifampicin (immediate release) formulation was used as a control formulation.

2.17.3 Study procedure

Volunteers were fasted overnight for at least 12 h, and abstained from alcohol, xanthine- and caffeine containing foods and fluids for 48 h prior to administration of the study formulation. The volunteers were not allowed to eat or drink water during the imaging period. The study protocol was designed to eliminate as many variables as possible that could affect gastroretention of the formulation in the stomach and/or gastric emptying of the formulations, e.g. to eliminate effects of food and beverages, and other factors (such as medication, disease, age). After administration of radio labelled dosage form, the subjects were imaged for 2 min. at a preset time (0, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 h) continuously for 120 second/view with a 10% window, centred to include the 140 keV photopeak of ^{99m}Tc.

2.17.4 Data analysis

Scintigrams were used to determine formulation activities in regions of interest (ROI). For each subject, an image that presented a full, clearly defined stomach shape was selected, and ROI were drawn around the stomach shape and anatomic marker. ROIs (relating to the stomach) were drawn manually on gamma images for each time point (of a fixed size for paired anterior and posterior images) using Gamma camera (Infinia, GE, India), and counts relating to ROIs were calculated using Xeleris software. All data was then corrected for radioactive decay, background and expressed in terms of corrected counts per cell within each ROI geometric means of counts in paired anterior and posterior images were calculated. All counts were corrected for background and decay. Gastric emptying of the formulations was expressed in terms of remaining relative counts (REL counts) in each ROI as a function of time.

Gastric emptying of the formulations was expressed in terms of remaining relative counts in each ROI as a function of time. Time at which half of the granules had left the stomach (T_{50}) were calculated and used in evaluating gastric-residence times.
The time to the onset of gastric emptying was determined as the time that showed hotspots of radioactivity leaving the stomach and entering the small intestine.

2.18 Stability studies of floating rifampicin tablet

The floating rifampicin tablets were prepared and subjected for stability studies. For this part of the study, the tablets were filled into empty hard gelatine capsule shells and were stored in tightly closed high density polyethylene (HDPE) containers and aluminium pack. The stability studies were carried out at room temperature and accelerated relative humidity conditions as per ICH guidelines. The accelerated relative humidity conditions were 40°C ± 2°C/75% RH ± 5% RH. The stability samples were analysed at 1, 2, 3 and 6 M. The assay, water content and dissolution studies of the floating rifampicin tablet was carried as per the methods described earlier. To ensure the equivalence in release profile of the stability samples with that of initial samples, the fit factor, similarity factor (f_2), was calculated. The formula used for calculating f_2 values is shown in Eq. (10):

$$f_2 = 50 \log\{[1+ 1/n\sum_{t=1}^{n}(R_t-T_t)]^{0.5} \text{ X } 100\} \qquad \text{--(10)}$$

2.19 Results and Discussion

2.19.1 Preliminary studies

During the preliminary studies, following approaches were adopted for the preparation of floating drug delivery system of rifampicin. These approaches included:

- **Rifampicin floating pellets using extrusion -spheronization**
- **Rifampicin floating pellets based on effervescent technique**
- **Rifampicin floating tablet**

For the preparation of floating pellets of rifampicin, various trials of rifampicin gastroretentive pellets were carried out using HPMC (HPMC K4M) / Methocoel A15LV, Sodium carboxy methyl cellulose and Sodium alginate by extrusion-spheronization technique. However, various process problems were observed. It was observed that a sticky mass was produced when moist blend containing HPMC (HPMC K4M) / Methocoel A15LV/ Sodium Carboxy Methyl cellulose/ Sodium alginate was passed through the extruder. In order to reduce the swelling tendency of the polymer matrix, amount of water was reduced. The reduction in the amount of water for granulation led to the production of dumbbell-shaped beads. Also, duration of floating for these rifampicin pellets were found to be very short. On the other hand, use of hydroalcoholic solution was ruled out due to higher solubility of rifampicin in alcoholic solvents, which led to the formation of watery mass during granulation.

To avoid these problems, a second approach was adopted. In this approach reservoir-type, multi-layer coated rifampicin pellets were designed as a FDDS. This FDDS was based on entrapment of generated gas within the polymeric film (Ichikawa *et.al*., 1991b; Sungthongjeen *et.al*., 2008). In this system, core matrix rifampicin pellet were prepared by extrusion spheronization followed by coating of the core pellet by an polymeric based (HPMC) inner effervescent layer (bicarbonate) and an outer polymeric membrane. Pellets thus obtained, showed a very low floating lag-time. However, floating duration of such pellets was very short. Also, high dose of rifampicin diminished the feasibility of increasing the amount of coating on the pellets.

Thus, it was decided to adopt another approach to develop FDDS of rifampicin. In view of the above problems, it was decided to formulate floating tablets of rifampicin, in place of rifampicin pellets.

2.19.2 Selection of variables

2.19.2.1 Formulation variables for floating rifampicin tablet

The approach used for the preparation of floating tablets of rifampicin was based on Hydrodynamically Balanced System (HBS) in combination with a gas generation component. For HBS system, the polymer selected were hydroxypropyl methylcellulose K4M, Carbopol 971P, while sodium bicarbonate was used as a gas generating component. It is expected that the initial lag time to float required by the tablet would be reduced with the help of gas generating component of the formulation. Meanwhile, the polymers will swell and reduce the density of the formulation less than gastric content resulting in floating of the tablet.

Preliminary studies were performed to develop a floating matrix tablet of rifampicin that showed a lag time to float <3 min. and duration of floating >5 h. Various trial formulations of floating tablets of rifampicin were prepared using HPMC K4M, Carbopol 971P, Methocoel A15LV, Sodium carboxy methyl cellulose, Sodium alginate etc. On the basis of prior studies on the floating properties of matrix tablets, it was concluded that combination of HPMC K4M and carbopol 971P are the best vehicle for the rifampicin floating tablet design. The rifampicin FDDS employed sodium bicarbonate as a gas forming agent, dispersed in a hydrogel matrix. The matrix was prepared by the combination of HPMC K4M and Carbopol 971P NF. During formation of the floating tablets, the evolving gas permeated through the matrix leaving gas bubbles or pores, which also increased the release rate of the active ingredient from the matrix. Amount of HPMC and carbopol was optimised using response surface optimised discussed later in this chapter in section 2.18.3. It was found during the preliminary trials that the use of sodium bicarbonate above 1% w/w level resulted in erosion of the matrix causing a reduced floating duration and rapid release of rifampicin. Thus, the amount of sodium bicarbonate was kept constant during trials at 1% w/w.

2.19.2.2 Process variables

The HPMC and carbopol were added extragranularly to avoid gelling and swelling of polymers at the formulation stage. The characteristic of rifampicin granules was kept constant throughout the experimentation and are enlisted in Table 13.

During preliminary experimentation it was found that floating ability of tablets was inversely proportional to the hardness of the tablet. Tablets prepared with hardness of ~ 80N were found to float for more than 7 h *in vitro*. Martínez *et.al*., 2008, ascribed this phenomena to the fact that tablets compacted at a lower pressure keep more entrapped air, decreasing the agglomerate density and allowing floating of the tablets. On the other hand, tablets compacted at higher pressure are less porous and display a density that does not allow the matrix to float. Hence, hardness of the tablet was kept constant at 80 Newtons.

Table 13. Evaluation of rifampicin granules of optimized composition

S.No.	Characteristic of granules	Observed values
1.	Bulk density (g/cm^3)	0.56 ± 0.13
2	Tapped density(g/cm^3)	0.89 ± 0.21
3.	Hausner ratio	1.75 ± 0.45
4.	Carr's Index	10.97± 1.79
5.	Angle of Repose (º)	28.75±2.01

2.19.3 Optimisation of floating rifampicin tablet

Mathematical relationship was generated between the factors (dependent variables) and responses (independent variables) using the statistical package Design-Expert for determining the levels of factors, which yield optimum formulation. A second order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 \quad \text{--(11)}$$

where b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; b_1 to b_5 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the coded levels of factors. The terms X_1X_2 and X_{2i} (i = 1 and 2) represent the interaction and quadratic terms, respectively.

The equation represents the quantitative effect of factors (X_1 and X_2) upon the responses (Y_1 and Y_2). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms

represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors. ANOVA was applied for estimating the significance of the model, at 5% significance level. A model is considered significant if the p-value (significance probability value) is less than 0.05.

2.19.3.1 Floating lag time

The floating lag time of the rifampicin floating tablet is an important characteristic. Ideally, the floating system should float within a few minutes after its contact with the gastric fluid (Iannuccelli *et.al*., 1998). The ANOVA analysis of floating lag time for the response, floating lag time, is presented in Table 14. A significant quadratic model ($p<0.05$), insignificant lack-of-fit test ($p>0.05$) and agreement of predicted and adjusted R-squared values allow data modelling (Table 14). Influence of amount of HPMC K4M and Carbopol can be explained by a mathematical relationship given in Eq. 12.

$$Y_1 = 3.39 - 0.33\ X_1 - 2.50\ X_2 - 0.083\ X_1^2 + 2.67\ X_2^2 + 0.00\ X_1X_2 \quad \text{--(12)}$$

It can concluded from the statistical analysis, that the amount of carbopol and its quadratic term were found to have significant influence on floating lag time of the floating rifampicin tablet (Table 14).

The response surface plot showing the influence of amount of HPMC K4M and amount of carbopol on floating lag time of rifampicin floating tablet is presented in Fig 14. It can be inferred from the graphical analysis and mathematical relationship (Fig 14 and Eq. 12) carbopol affects the floating lag time in almost a negative and a curvilinear fashion. On increasing the amount of carbopol, the floating lag time of floating rifampicin pellet is decreased.

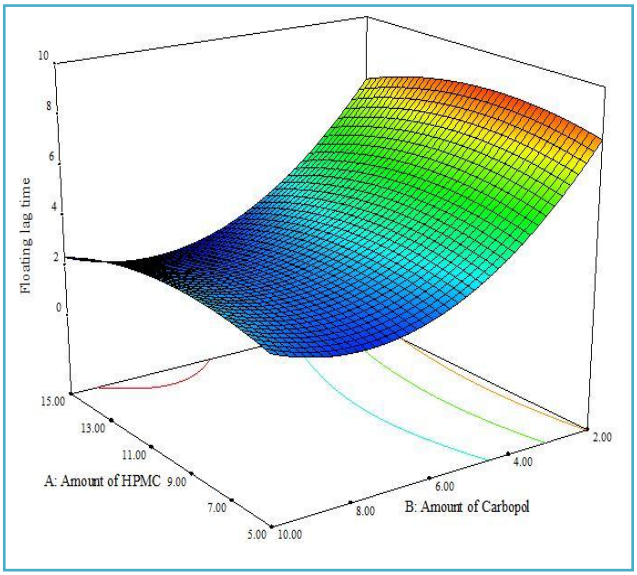
This might be due to the fact that carbopol has a tendency of rapid uptake of water. This results in swelling of carbopol network and holding of water inside its microgel network (Singla *et.al.,* 2000). The rapid swelling of the matrix results in the decrease in density of the matrix, imparting to rapid floatability to the formulation. Since the swelling of HPMC is slower in comparison to carbopol, effect of carbopol in decreasing the floating lag time is more pronounced and significant.

Table 14. ANOVA results for floating lag time

Floating lag time	
Source	*p* value
Model	**0.0241**
X_1	0.4022
X_2	**0.0053**
X_1^2	0.2547
X_2^2	**0.0205**
X_1 X_2	1.000
R^2	0.9622
Adjusted R^2	0.8993

Significant terms are shown in bold type

Fig 14. Response surface plot showing the influence of amount of HPMC K4M and amount of carbopol on floating lag time of rifampicin floating tablet



2.19.3.2 Floating duration

The major objective of floating drug delivery system is to prolong the gastric retention of the dosage form in the stomach *via* floating the formulation on the gastric content. The floating duration of the rifampicin floating tablet is thus a very important characteristic. The ANOVA analysis of floating duration for the responses is presented in Table 15. Significant quadratic model ($p<0.05$), insignificant lack-of-fit test ($p>0.05$) and agreement of predicted and adjusted R-squared values allow data modelling (Table

15). Influence of amount of HPMC K4M and Carbopol can be explained by a mathematical relationship described in Eq. 13.

$$Y_2 = 6.78 + 1.28\ X_1 + 1.33\ X_2 - 0.62\ X_1^2 - 0.97\ X_2^2 + 0.12\ X_1X_2 \quad \text{--(13)}$$

It can be concluded from the statistical analysis that the amount of HPMC and amount of carbopol have a significant influence on floating duration of the floating rifampicin floating tablet (Table 15). It can be inferred from the graph and mathematical relationship that both the factors have a positive effect on the floating duration of rifampicin FDDS. This might be attributed to the fact that HPMC and carbopol both has tendency of rapid hrdration and uptake of water and thus forming hydrogel. The response surface plot for the influence of amount HPMC K4M and amount of carbopol on floating duration of rifampicin FDDS is shown in Fig 15. It can be seen in Fig 15. that amount of HPMC and carbopol affects the floating duration in almost a positive and a curvilinear fashion.

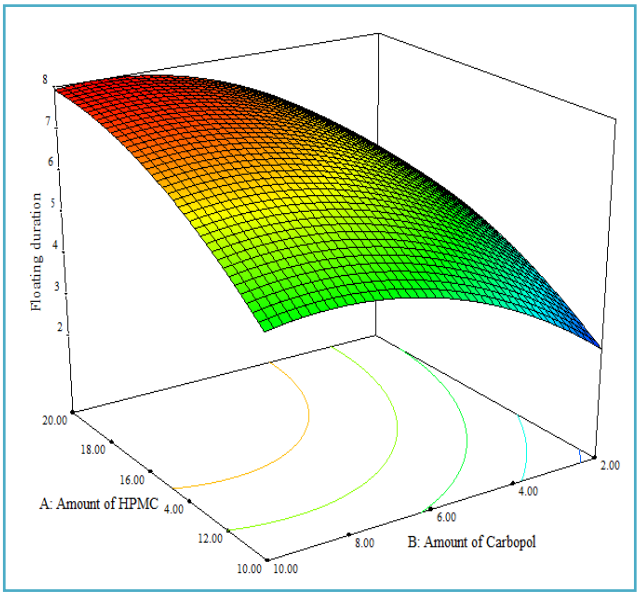
In the present investigation, the gastric floating system employed sodium bicarbonate as a gas forming agent dispersed in hydrogel matrix. After reacting with hydrochloride acid, sodium bicarbonate creates carbon dioxide whose bubbles were entrapped in the hydrogel matrix of the tablet causing the tablet to float for more than 6 h *in vitro*. This is in contrast to earlier reports of Li *et.al.*, 2003. However, Xiaoqiang *et.al.*, 2006, successfully demonstrated development of HPMC and Carbopol based floating matrix dosage form for phenylproplamine hydrochloride in human volunteers.

Table 15. ANOVA results for floating duration

Floating duration	
Source	*p* value
Model	**0.0047**
X_1	**0.0021**
X_2	**0.0019**
X_1^2	0.0690
X_2^2	**0.0223**
$X_1\ X_2$	0.4838
R^2	0.9875
Adjusted R^2	0.9665

Significant terms are shown in bold type

Fig 15. Response surface plot showing the influence of amount HPMC K4M and amount of carbopol on floating duration of rifampicin floating tablet



2.19.3.3 *In vitro* drug release

FDDS is expected to release drug for a prolonged period of time in stomach. Time taken for 80% of drug to be released was selected as an indicator of *in vitro* drug release from the floating rifampicin tablet. The ANOVA analysis of floating duration is presented in Table 16. A significant second order model ($p<0.05$), agreement of predicted and adjusted R-squared values allow data modelling (Table 16). Influence of amount of HPMC K4M and Carbopol can be expressed by a mathematical relationship shown in Eq. 14.

$$Y_3 = 5.61 + 2.12\ X_1 + 0.23\ X_2 - 0.50\ X_1X_2 \quad \text{--(14)}$$

The response surface plot for the influence of amount HPMC K4M and amount of carbopol on $t_{80\%}$ from rifampicin FDDS is shown in Fig 16.

Statistical analysis indicates that the amount of HPMC and its interaction term have significant influence on the floating lag time of the FDDS of rifampicin (Table 16). The response surface plot showing the influence of amount HPMC K4M and amount of carbopol on $t_{80\%}$ is presented in Fig 16. It can be inferred from the graph and mathematical relationship (Fig 16 and Eq. 14), that amount of HPMC affects the drug release duration in a near linear, ascending trend. On increasing the amount of HPMC, the amount of drug release is prolonged from the floating rifampicin tablet is increased. This can be attributed to the fact that HPMC with higher viscosity results in thicker gel layer formation, which retards the drug release. Proportions of HPMC modify the release

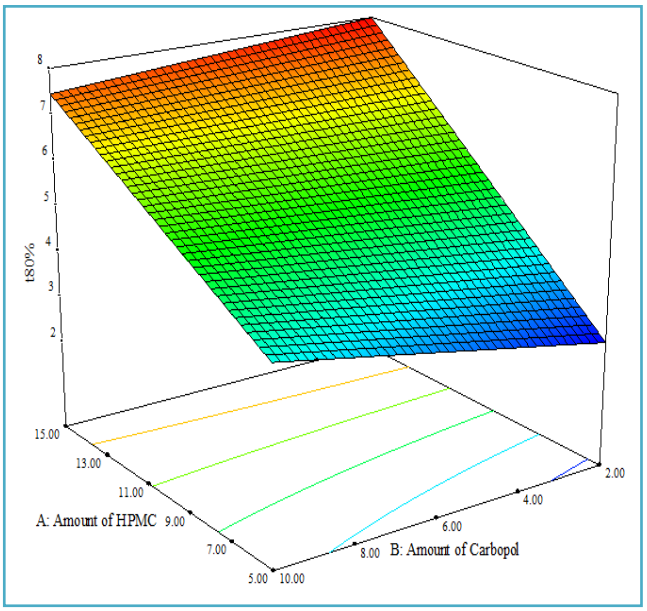
mechanism from diffusion toward a relaxation and erosion controlled process. The restriction of drug release is associated with an extended time of matrix exposure to the dissolution medium to release a given quantity of the drug. Consequently, every release restriction in the rifampicin / HPMC system is associated to a higher degree of matrix hydration before a given quantity of the drug is released. It means a greater contribution of matrix relaxation and erosion processes to predominant release mechanism. Moreover, by increasing water content, the diffusion coefficient of the drug increases substantially (Siepmann *et.al*., 2002).

Table 16. ANOVA results for *in vitro* drug release

***In vitro* drug release**	
Source	p value
Model	**< 0.0001**
X_1	**< 0.0001**
X_2	0.0736
X_1 X_2	**0.0109**
R^2	0.9888
Adjusted R^2	0.9820

Significant terms are shown in bold type

Fig 16. Response surface plot showing the influence of amount HPMC K4M and amount of carbopol on $t_{80\%}$



2.19.3.4 Validation of multiple response optimization model

In order to assess the reliability of the developed mathematical model, formulations corresponding to the optimum composition and two additional random compositions covering the entire range of experimental domain were prepared.

For optimization of formulation, constraints were applied and the optimum formulation was calculated. Thus, to obtain optimum floating rifampicin tablet, it is desirable to have floating lag time (Y_1<3 min.) with maximum floating duration (Y_2 > 6 h) and a prolonged and maximum drug release for more than 6 h (Y_3, $t_{80\%}$~ 6 h). The formulation containing amount of HPMC, X_1 = 15.44% w/w and amount of carbopol, X_2 = 7.82% w/w, was found to be optimum. Table 17 enlists the compositions of the checkpoints, their predicted values of all the response variables, and the percentage error in prognosis.

The above-mentioned optimized formulation was evaluated for all the parameters and showed low values of prediction percentage error indicating that the predicted and observed values are in good agreement (Table 17). Thus, the lower magnitudes of the error in current study indicate the robustness of the model used for the optimization of rifampicin floating formulation. It can also be concluded that, for the optimum formulation, the results of the physical evaluation and tablet assay was found to be within limits.

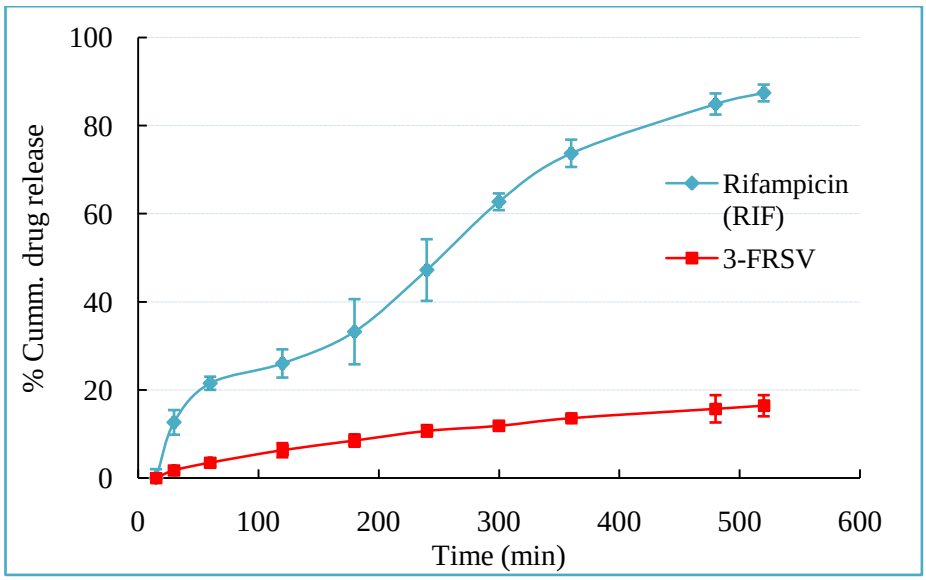
Rifampicin release profile from the optimised rifampicin floating formulation is shown in Fig 17. It can be seen that at the end of 520 min. more that 80% of the drug was released while 3-FRSV formed during this duration was ~16%. It can be seen from the graph that rifampicin was gradually released (~80%) over a period of 520 min. along with sufficient *in vitro* floating duration (> 6 h).The floating of the tablet was attributed to the presence of HPMC K4M along with carbopol and to gas formation resulting from the chemical reaction between $NaHCO_3$ and hydrochloric acid. Simultaneously, the gelling property of HPMC K4M and carbopol is responsible for sustaining drug release from the matrix tablet (Xiaoqiang *et.al*., 2006). The drug release from swellable and erodible hydrophilic matrices can be attributed to polymer dissolution, drug diffusion through the gel layer, or a combination of both (Gao *et.al*., 1995).

Table 17. The experimental and predicted values for all the eight responses (Y_1 to Y_4) along with percentage prediction error* observed for optimum formulation (A) and random formulation (B and C)

Formulation ($X_{1\%}$, X_2%)	Response	Predicted Value	Expected value	% PE*
A **15.44, 7.82** ***(Optimum)***	Y_1	2.76	2.70	-2.22
	Y_2	6.14	6.50	5.54
	Y_3	5.89	6.00	1.84
B **19.63, 5.17**	Y_1	2.99	3.20	6.56
	Y_2	7.71	7.80	1.15
	Y_3	7.62	7.50	-1.60
C **10.24, 7.39**	Y_1	2.40	2.20	-9.09
	Y_2	3.57	3.50	-2.00
	Y_3	3.84	3.90	1.54

***%PE was calculated using the formula (Experimental Value – Predicted Value) / Experimental Value x 100.

Fig 17. *In vitro* release profile of rifampicin from optimized floating rifampicin formulation along with its degradation product (3-FRSV)



2.20 *In vivo* gastroretention using gamma-scintigraphic study

To confirm the *in vivo* performance of the rifampicin floating formulation gamma-scintigraphic studies were carried out in healthy human volunteers. The representative gamma-scintigraphic images of rifampicin floating formulation in human volunteers are

shown in Fig 18. While, gamma-scintigraphic images of immediate release rifampicin is shown in Fig 19.

It can be clearly deduced from the gamma-scintigraphic images (Fig 18) that, at t = 0 min., immediately after ingestion, the capsule containing the floating tablet was located on the surface of the gastric fluid. At t= 0 min., as the floating tablet was confined in the capsule, the radioactivity could be visualised as a single hotspot. At t = 60 min., the floating tablet can be seen as localized in the upper part of the stomach. During this duration, the capsule opened and the floating tablets began to be dispersed in the stomach. This can be seen as radioactivity dispersed throughout the stomach.

After 6 h i.e., t = 360 min., floating tablet can be visualised in the stomach as two hotspots, indicating that the floating tablet has disintegrated into two halves. However, both the halves are still in stomach.

In contrast, for immediate release capsule, at t = 0 min. in Fig 19, immediately after ingestion, the capsule can be located in the stomach as a single hotspot. After 1 h i.e., t = 60 min., the capsule opened up and began to get dispersed in the stomach, which seen as radioactivity throughout the stomach. After 2 h, it can be clearly seen that formulation has been emptied from the stomach and moved ahead into the intestine as well as it has disintegrated into two, which are seen as two hotspots. Between 150 min. to 360 min., immediate release formulation has completely left the stomach.

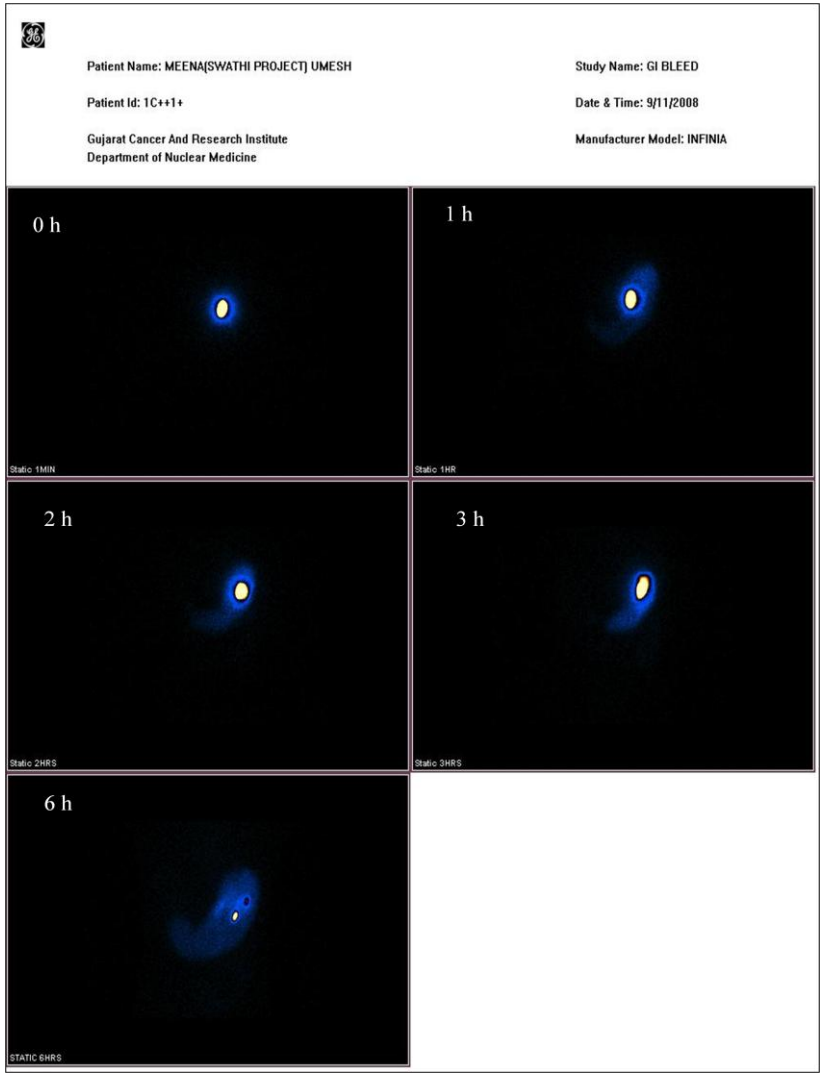
These results clearly indicate that floating rifampicin tablet is retained for a longer time than the conventional rifampicin formulation. The retention time of floating rifampicin tablet in human volunteer confirms that formulation remains in the stomach was ~6 h.

The comparative gastric emptying of floating rifampicin formulation and conventional release rifampicin formulation is shown in Fig 20. T_{50} for gastric emptying was calculated graphically, Fig 20. T_{50} for gastric emptying from gamma-scintigraphic study of floating rifampicin formulation was found to be around 345 min. While, the marketed rifampicin (immediate release) formulation showed $T_{50,}$ for gastric emptying, of around 90 min. The prolonged retention of floating tablet of rifampicin can be attributed to the presence of HPMC and Carbopol in the formulation. Both, HPMC and Carbopol, has rapid water uptake tendency followed by swelling of the polymer. As a result of the swelling of the polymers, the density of the formulation decreases which imparts floating behavior to the formulation.

Fig 18. A representative gamma scintigraphic image of floating rifampicin formulation in human volunteer

a). Gamma scintigraphic image of rifampicin floating formulation

(b). Gamma scintigraphic image corrected for background count in the region of interest



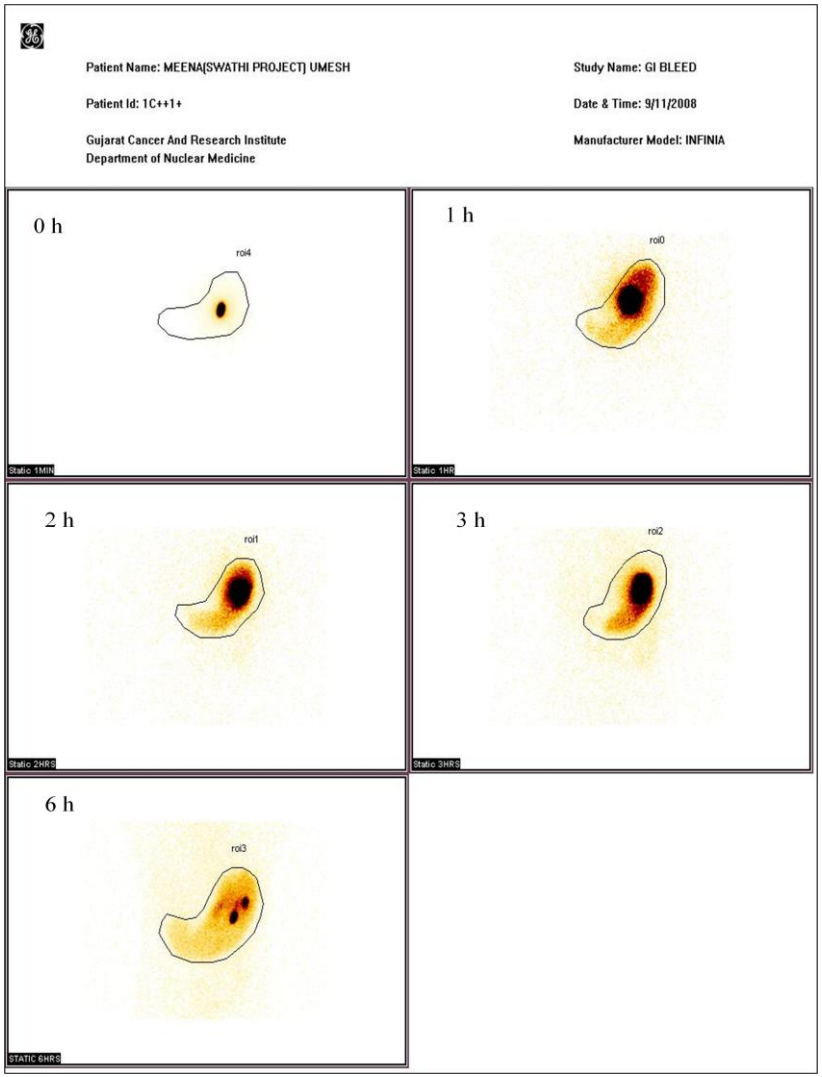
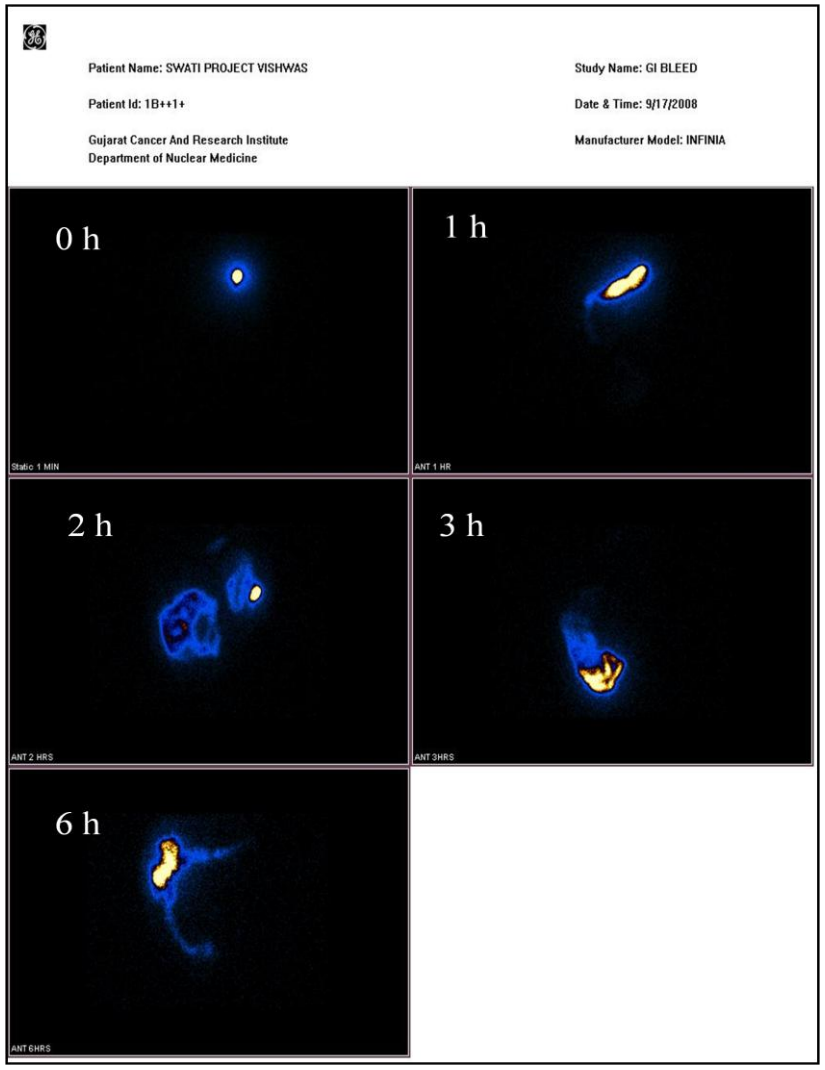


Fig 19. A representative gamma scintigraphic image of immediate release rifampicin formulation in human volunteer

a). Gamma scintigraphic image of immediate release rifampicin



(b).Gamma scintigraphic image corrected for background count in the region of interest

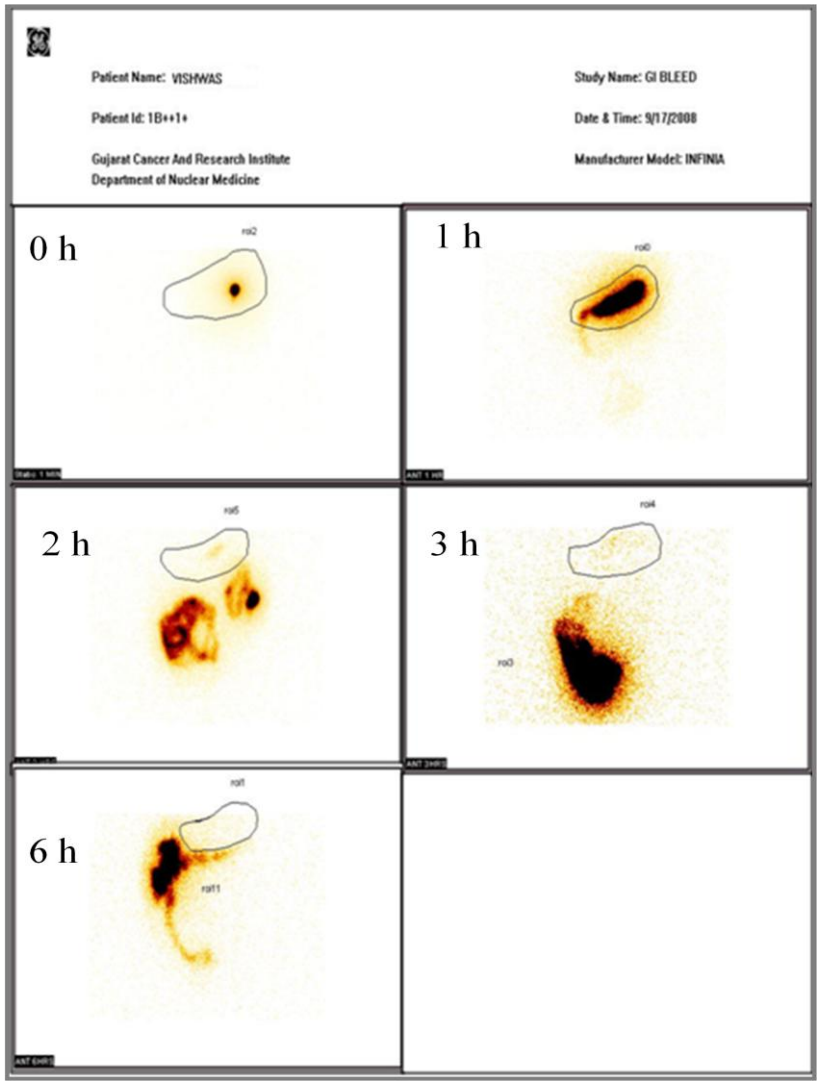
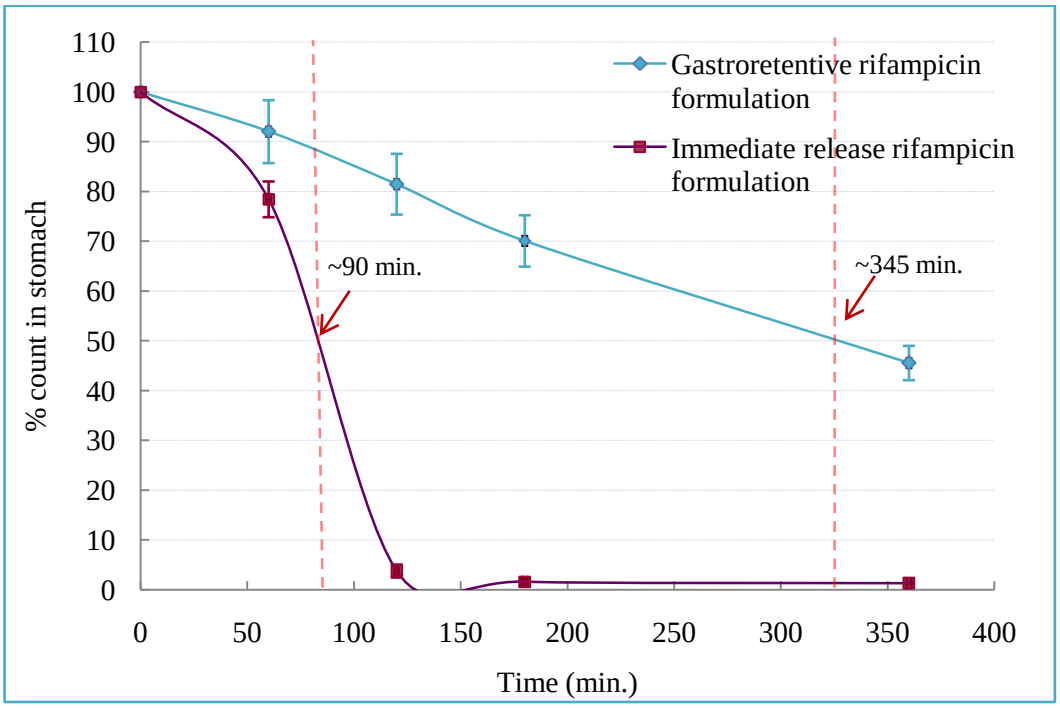


Fig 20. Comparative gastric emptying of floating rifampicin formulation and conventional release rifampicin formulation



2.21 Stability studies

The quality of a drug product changes with time under the influence of environmental factors such as temperature, humidity and light. The purpose of stability testing is to investigate these changes, to establish a shelf life for the drug product and to recommend storage conditions. This will be applicable to all future batches of the tested drug product manufactured and packaged under similar circumstances (Lusina *et.al*., 2005). Floating rifampicin tablets were subjected to stability study at room temperature and at accelerated stability conditions. The samples were withdrawn at 1M, 2 M, 3 M and at 6 M and evaluated for water content, drug content and release profile.

Drug content and water content of the rifampicin floating tablet on stability are enlisted in Table 18. It was found that drug content of stability samples varied between 98.99-100.35% and water content ranged between 3.65- 4.54% w/w. It can be concluded that water content and drug content did not change significantly on subjecting the formulation to stability studies. Data shows that rifampicin floating tablets are stable.

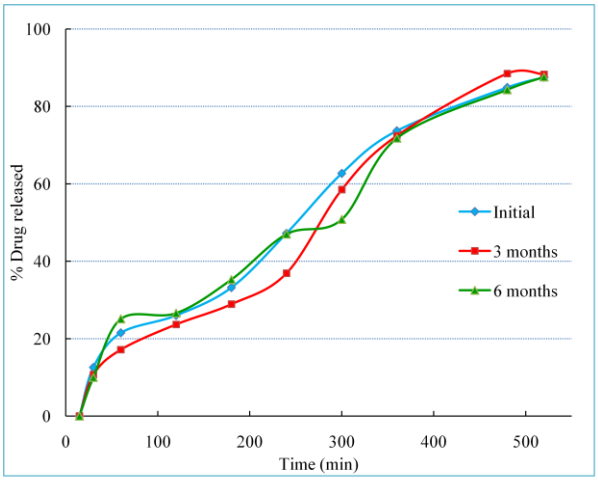
Table 18. Assay and water content of stability samples of rifampicin floating tablet at 1 M, 2 M, 3 M and 6 M

		40°C ± 2°C/75% RH ± 5% RH		At room temperature	
		HDPE	Aluminium strip	HDPE	Aluminium Strip
Assay (%)	**1M**	100.35	100.14	100.16	99.65
	2M	100.07	99.99	98.65	98.99
	3M	99.67	100.03	98.78	100.18
	6M	99.43	99.89	99.79	100.01
Water Content (%w/w)	**1M**	4.01	4.25	3.65	4.08
	2M	4.54	4.15	3.99	4.37
	3M	4.43	4.22	4.17	4.20
	6M	4.38	4.18	4.29	4.05

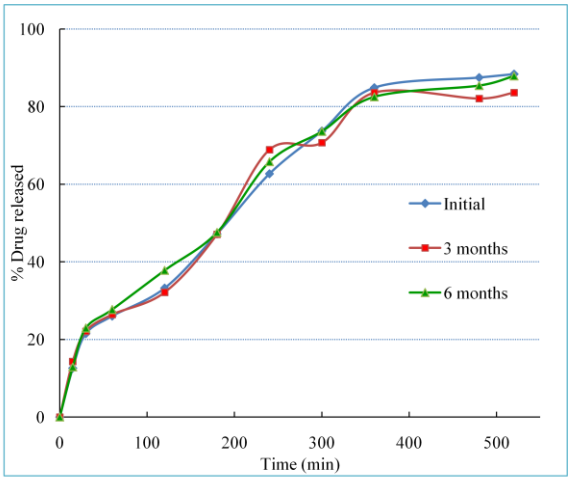
The release profile of rifampicin floating tablet subjected to stability studies are shown in Fig 21.It can be seen that the release profile of sample at 40°C ± 2°C/75% RH ± 5% RH and at room temperature, does not change till 6 M of storage at accelerated condition and at room temperature. To statistically establish the equivalence between the release profiles, a fit factor, f_2 similarity factor was applied. It was found that f_2 similarity factor of floating rifampicin tablet after 6M accelerated stability study and room temperature, when compared with their initial values, was >80. The f_2 value above 50 is indicative of statistical similarity of the two release profile.

Based on f_2 value, it can be concluded that there was no significant change in the dissolution profile of rifampicin floating tablet on stability profile.

Fig 21. Dissolution profile of initial, 3 M and 6 M stability samples of rifampicin floating tablet



a. Floating rifampicin tablets at 40 °C/75% RH



b. Floating rifampicin tablets at RT

2.22 Conclusions

Floating tablets of rifampicin were prepared and evaluated and following conclusions can be made.

- Floating rifampicin tablets was prepared using a combination of HPMC K4M and carbopol. The amount of HPMC K4M and carbopol was optimised using response surface optimization. The optimized formulation gave sufficient duration of floating (>520 min.) and a shorter duration of floating lag time (< 3 min.). These tablets were also found to have desirable physical properties (friability < 1%, drug content: 100.15% ± 2.15).
- The *in vivo* performance of the floating rifampicin tablet was evaluated using gamma-scintigraphy technique. The gamma-scintigraphic studies of floating rifampicin formulation were carried out in human volunteer. Gamma-scintigraphic studies reveal that rifampicin floating formulation remains in the stomach for ~6 h. T_{50} for gastric emptying from gamma-scintigraphic study of floating rifampicin formulation was found to be around 345 min. While, the marketed rifampicin (immediate release) formulation showed $T_{50,}$ for gastric emptying, of around 90 min.
- Floating rifampicin tablet was subjected to accelerated stability conditions, 40°C ± 2°C/75% RH ± 5% RH and at room temperature for 6 M. The data shows that floating rifampicin tablets are stable at 40°C ± 2°C/75% RH ± 5% RH (Assay 98.99-100.35%). Floating rifampicin tablets showed no significant change in the dissolution profile as indicated by their respective f_2 similarity factor > 90.

References

Acocella, G., Nicolis, F.B., Lamarina, A., 1967. A study of the kinetics of rifampicin in man. Chemotherapia 5, 87.

Acocella, G., Pagani, V., Marchetti, M., Barconi, C.G., Nicolis, F.B., 1971. Kinetic studies on rifampicin. Chemotherapy 16, 365-370.

Acocella, G., 1978. Clinical pharmacokinetics of rifampicin. Clin. Pharmacokinet. 3, 108-127.

Almeida-Prieto, S., Blanco-Méndez, J., Otero-Espinar, F.J. 2007. Microscopic image analysis techniques for the morophological charterization of pharmaceutical particles: Influence of the software and the factor algorithms used in the shape factor estimation. Eur. J. Pham. Biopharm. 67, 766-776.

Amighi, K., Timmermans, J., Puigdevall, J., Baltes, E., Moes, A.J., 1998. Peroral sustained-release film-coated pellets as a means to overcome physicochemical and biological drug-related problems. Part 1. *In vitro* development and evaluation. Drug Dev. Ind. Pharm. 24, 509–515.

Bardonnet, P.L., Faivre, V., Pugh, W.J., Piffaretti, J.C., Falson, F., 2006. Gastroretentive dosage forms: Overview and special case of Helicobacter pylori. J. Control. Release 111, 1 – 18.

Baumgartner, S., Kristl, J., Vrecer, F., Vodopivec, P., Zorko, B., 2000. Optimisation of floating matrix tablets and evaluation of their gastric residence time, Int. J. Pharm. 195 (1–2), 125– 135.

Bhutani, H., Mariappan, T.T., Singh, S., 2004. A study on the physical and chemical stability of anti-tuberculosis fixed-dose combination (FDC) products under accelerated climatic conditions. Int. J. Tuber. Lung Dis. 8, 1073-1080.

Burman, W.J., Gallicano, K., Peloquin, C., 2001. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin. Pharmacokinet. 40, 327 41.

Chen, G.L., Hao, W.H., 1998. *In vitro* performance of floating sustained release capsule of verapamil, Drug Dev. Ind. Pharm. 24 (11), 1067– 1072.

Chopra, R., Newton, J.M., Alderborn, G., and Podczeck, F. 2001. Preparation of pellets of different shape and their characterization. Pharm. Develop. Technol. 6, 495-503.

Douglas, J., McLeod, M., 1999. Pharmacokinetic factors in the modern drug treatment of tuberculosis. Clin. Pharmacokinet. 37, 127-46.

Ellard, G.A., Fourie, P.B., 1999. Rifampicin bioavailability: a review of its pharmacology and the chemotherapeutic necessity for ensuring optimal absorption. Int. J. Tuber. Lung Dis., 3(11), S301–S308.

Engelberg-Kulka,H., Sat, B., Reches, M., Amitai, S., Hazan, R., 2004. Bacterial programmed cell death systems as targets for antibiotics. Trends Microbiol. 12, 66 70.

Erni, W., Held, K., 1987. The hydrodynamically balanced system: a novel principle of controlled drug release, Eur. Neurol. 27 (Suppl. 1), 21– 27.

Fábregas, J.L., Claramunt, J., Cucala, J., Pous, R., Siles, A., 1994. In vitro testing of an antiacid formulation with prolonged gastric residence time (Almagate Flot-Coat), Drug Dev. Ind. Pharm. 20, 1199– 1212.

Gao, P., Nixon, P.R., Skoug, J.W., 1995. Diffusion in HPMC gels, II: prediction of drug release rates from hydrophilic matrix extended release dosage forms. Pharm Res. 12, 965-971.

Ghebre-Sellassie, I., 1989. Pellets: A general overview. In Ghebre-Sellassie, I (ed.), Pharmaceutical Pelletization technology. Marcel Dekker, Inc., New York, USA, Vol. 37, pp. 1-13.

Hellén, L., Yliruusi, J., 1993. Process variables of instant granulator and spheroniser: III. Shape and shape distributions of pellets. Int. J. Pharm. 96, 217-223.

Howard, M.A., Neau, S. H., Marvin, J.S., 2006. PEO and MPEG in high drug load extruded and spheronized beads that are devoid of MCC. Int. J. Pharm. 307, 66-76.

Hwang, S.J., Park, H., Park, K., 1998. Gastric retentive drug-delivery systems, Crit. Rev. Ther. Drug Carr. Syst. 15 (3), 243– 284.

Iannuccelli, V., Coppi, G., Bernabei, M.T., Cameroni, R., 1998. Air compartment multiple-unit system for prolonged gastric residence. Part I. Formulation study, Int. J. Pharm. 17, 47–54.

Ichikawa, M., Kato, T., Kawahara, M., Watanabe, S., Kayano, M., 1991a. A new multiple-unit oral floating dosage system: II. In vivo evaluation of floating and sustained-release characteristics with p-aminobenzoic acid and isosorbide dinitrate as model drugs. J. Pharm. Sci. 80 (12), 1153–1156.

Ichikawa, M., Watanabe, S., Miyake, Y., 1991b. A new multiple-unit oral floating dosage system: I. Preparation and in vitro evaluation of floating and sustained-release characteristics, J. Pharm. Sci. 80 (11), 1062– 1066.

ICH harmonised tripartite guideline, 2003. Stability testing of new drug substances and products Q1a (R2), 4th version.

Immanuel, C., Gurumurthy, P., Ramachandran, G., Venkatesan, P., Chandrasekaran, V., Prabhakar, P., 2003. Bio-availability of Rifampicin following concomitant administration of Ethambutol or Isoniazid or Pyrazinamide or a combination of the three drugs. Indian J. Med. Res. 118, 109-114.

IUTALD/ WHO. A joint statement, 1999. Assuring bioavailability of fixed-dose combinations of anti-tuberculosis medications. Int. J. Tuber. Lung Dis. 3(11), S282–S283.

Jack, D.B., 1992. Handbook of Clinical Pharmacokinetic data. Jack, D.B. (Ed.), Macmillian Publishers Ltd., pp. 62-63.

Jansen, E.N., Meerwaldtt, J.D., 1990. Madopar HBS in nocturnal symptoms of Parkinson's disease, Adv. Neurol. 53, 527– 531.

Koo, O.M.Y.K., and Heng, P.W.S. 2001. The influence of microcrystalline cellulose grade on shape and shape distributions of pellets produced by extrusion- spheronization. Chem. Pharm. Bull. 49, 1383-1387.

Kebrele, H., 1970. Physicochemical factors of drugs affecting absorption, distribution, and excretion. Acta Pharma. 30-47.

Kagan, L., Hoffman, A., 2008. Systems for region selective drug delivery in the gastrointestinal tract: biopharmaceutical considerations. Expert Opin. Drug Deliv. 5(6), 681-692.

Kleinebudde, P., 1994. Shrinking and swelling properties of pellets containing microcrystalline cellulose and low substitute hydroxypropylcellulose. Int. J. Pharm. 209– 219.

Laserson, K.F., Kenyon, A.S., Kenyon, T.A., Layloff, T., Binkin, N.J., 2001. Substandard tuberculosis drugs on the global market and their simple detection. Int. J. Tuber. Lung Dis. 5, 448-454.

Li, S., Lin, S., Daggy, B.P., Mirchandani, H.L., Chien, Y.W, 2003. Effect of HPMC and Carbopol on the release and floating properties of Gastric Floating Drug Delivery System using factorial design. Int. J. Pharm. 253, 13–22.

Loos, U., Musch, E., Jensen, J.C., Mikus, G., Schwabe, H.K., Eichelbaum, M., 1985. Pharmacokinetics of oral and intravenous rifampicin during chronic administration. Klinische Wochenschrift 63, 1205-1211.

Lusina, M., Cindri´c, T., Tomai´c, J., Peko, M., Pozai´c, L., Musulin, N., 2005. Stability study of losartan/hydrochlorothiazide tablets. Int. J. Pharm. 291, 127–137.

Luyen, L.T., Huyen, H.T.K., Sang, T.V., Huong, N.T.L., 2005. Effects of Isoniazid and Pyrazinamide on bio-availability of Rifampicin when co-administered Rifampicin-Isoniazid-Pyrazinamide. TCNCYH. 36, 12-17.

Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of Rifampicin and Isoniazid (alone and their combination) in the rat. Int. J. Tuber. Lung Dis. 7, 797-803.

Martínez, I.J., Barreda, T.Q., Robles, L.V., 2008. Sustained delivery of captopril from floating matrix tablets. Int. J.Pharm.362, 37–43.

Michaels, A.S., 1974. Drug delivery device for self actuated mechanism for retaining device in selected area, US patent 3786813.

Michaels, A.S., 1975. Integrated device for administering beneficial drug at programmed rate, US patent 3901232.

Mitra, S.B., 1984. Sustained release oral medicinal delivery devices. US patent 4,451,260.

Moes, A.J., 1993. Gastroretentive dosage forms. Crit. Rev. Ther. Drug Carr. Syst. 10 (2), 143–195.

Nahata, M.C., Fan-Hovard, P., Barson, W.J., Bartkowski, H.M., Kosnik, E.J., 1990. Pharmacokinetics, cerebrospinal fluid concentration and safety of intravenous rifampicin in pediatric patients undergoing shunt placements. Eur. J. Clin. Pharmacol. 38, 515-517.

Newton, J.M., Chapman, S.R., Rowe, R.C., 1995. The influence of process variables on the preparation and properties of spherical granules by the process of extrusion and spheronisation. Int. J. Pharm. 120, 101-109.

Newton, J.M., Pinto, M.R., Podczeck, F. 2007. The preparation of pellets containing a surfactant or a mixture of mono- and di-glycerides by extrusion/spheronization. Eur. J. Pharm. Sci. 30, 333-342.

Oth, M., Franz, M., Timmermans, J., Moes, A., 1992. The bilayer floating capsule: a stomach-directed drug delivery system for misoprostol, Pharm. Res. 9 (3), 298– 302.

Panchagnula, R., Kaur, K.J., Singh, I., Kaul, C.L., 1999. The WHO simplified study protocol in practice: investigation of combined formulations supplied by the WHO. Int. J. Tuber. Lung Dis. 3, S336– S342.

Paterakis, P.G., Korakianiti, E.S., Dallas, P. P., Rekkas, D.M., 2002. Evaluation and simultaneous optimization of some pellets characteristics using a 33factorial design and the desirability function. Int. J. Pharm. 26, 953-963.

Petri, W.A., 2001. Antimicrobial Agents. In: Hardmann, J.G., Limbird, L.E., Gilman, A.G. (Eds.), The Goodman and Gilman's: The pharmacological basis of therapeutics. MacGraw Hill Medical Publishing division, New York, 10th ed., pp. 1273-1294.

Podczeck, F., Rahman, S.R., Newton, J.M., 1999. Evaluation of a standardised procedure to assess the shape of pellets using image analysis. Int. J. Pharm. 192, 123–138.

Rastogi, N., Goh, K.S., Bryskier, A., Devallois, A., 1996. *In vitro* activities of levofloxacin used alone and in combination with first- and second-line antituberculous drugs against *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 40, 1610-1616.

Reddy, L.H., Murthy, R.S., 2002. Floating dosage systems in drug delivery, Crit. Rev. Ther. Drug Carr. Syst. 19 (6), 553–585.

Reynolds, J.E.F., 1993. Martindale- The Extra Pharmacopeia. The council of the Royal Pharmaceutical Society of Great Britain, 30th ed., pp. 574.

Ritschel, W.A., 1991. Targeting in the gastrointestinal tract: new approaches, Methods Find. Exp. Clin. Pharmacol. 13 (5), 313– 336.

Sankar, R., Sharda, N., Singh, S., 2003. Behaviour of decomposition of Rifampicin in the presence of Isoniazid in the pH range 1-3. Drug Dev. Ind. Pharm. 29, 733-738.

Savale, S.S., 2003. Dissolution study and its correlation with bioavailability of the drugs. Ph.D. Thesis, Gujarat University, Ahmedabad.

Sato, Y., Kawashima, Y., Takeuchi, H., Yamamoto, H., 2004. In vitro and in vivo evaluation of riboflavin-containing microballoons for a floating controlled drug delivery system in healthy humans, Int. J. Pharm. 275 (1– 2), 97– 107.

Sensi, P., Gressi, G.G., 1996. Antimycobacterial Agents. In: Wolff, M.E. (Ed.) Burger's Medicinal chemistry and drug discovery. Wiley-Interscience Publication, New York, 5th ed., vol 2., pp. 575-635.

Sheth, P.R., Tossounian, J., 1984. The hydrodynamically balanced system (HBS): A novel drug delivery system for oral use, Drug Dev. Ind. Pharm. 10, 313–339.

Shishoo, C.J., Shah, S.A., Rathod, I. S., Savale, S. S., Kotecha, J. S., Shah, P.B., 1999. Stability of rifampicin in dissolution medium in presence of Isoniazid. Int. J. Pharm. 190, 109–123.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., 2001. Impaired Bioavailability of Rifampicin from its Fixed Dose Combination (FDC) Formulations with Isoniazid, Indian J. Pharm. Sci., 63(6), 443-449.

Siepmann, J., Streubel, A., Peppas, N.A., 2002. Understanding and predicting drug delivery from hydrophilic matrix tablets using the "sequential layer" model. Pharm. Res. 19, 306–314.

Siegler, D.I., Bryant, M., Burley, D.M., Citron, K.M., Standen, S.M., 1974. Effect of meals on rifampicin absorption. Lancet 2, 197-198.

Singh, B.N., Kim, K.H., 2000. Floating drug delivery systems: an approach to oral controlled drug delivery *via* gastric retention, J. Control. Rel. 63 (3), 235– 259.

Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.

Singh, S., Mariappan, T.T., Sharda, N., Kumar, S., Chakraborti, A.K., 2000a. The reason for an increase in decomposition of Rifampicin in the presence of Isoniazid under acid conditions. Pharm. Pharmacol. Commun. 6, 405- 410.

Singh, S., Mariappan, T.T., Sharda, N., Singh, B., 2000b. Degradation of Rifampicin, Isoniazid and Pyrazinamide from prepared mixtures and marketed single and combination products under acid conditions. Pharm. Pharmacol. Commun. *6*, 491-494.

Singh, S., Mohan, B., 2003. A pilot stability study on anti-tuberculosis four drug fixed dose combination products. Int. J. Tuber. Lung Dis. 7, 298-303.

Singla, A.K., Chawla, M., Singh, A., 2000. Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review. Drug Dev. Ind. Pharm. 26, 913–924.

Sousa, A., Podczeck, F., and Newton, J.M. 2002. Factors influencing the physical characteristics of pellets obtained by extrusion-spheronization. Int. J. Pharm. 232, 91-106.

Souto, C., Rodriguez, A., Parajes, S., Martinez-Pacheco, R., 2005. A comparative study of the utility of two superdisintegrants in microcrystalline cellulose pellets prepared by extrusion-spheronization. Eur. J. Pharm. Biopharm. 61, 94–99.

Steckel, H., Mindermann-Nogly, F., 2004. Production of chitosan pellets by extrusion/ spheronization. Eur. J. Pharm. Biopharm. 57, 105-114.

Stithit, S., Chen, W., Price, J.C., 1998. Development and characterization of buoyant theophylline microspheres with near zero order release kinetics, J. Microencapsul 15, 725– 737.

Sungthongjeen, S., Sriamornsak, P., Puttipipatkhachorn, S., 2008. Design and evaluation of floating multi-layer coated tablets based on gas formation Eur. J. Pharm. Biopharm. 69 (1), 255-263.

Sungthongjeen, S., Paeratakul, O., Limmatvapirat, S., Puttipipatkhachorn, S., 2006. Preparation and *in- vitro* evaluation of a multiple-unit floating drug delivery system based on gas formation technique. Int. J. Pharm. 324,136–143.

Teuinssen, M.W., Bakker, W., Meerburg-Van der Torren, J.E., Breimer, D.D., 1984. Influnence of rifampicin treatment on antipyri ne clearance and metabolite formation in patients with tuberculosis. Br. J. Clin. Pharmacol. 18, 701-706.

US Pharmacopoeia 30/NF25, 2007a. US Pharmacopoeial Convention, Rockville, MD, pp. 3128.

US Pharmacopoeia 30/NF25, 2007b. US Pharmacopoeial Convention, Rockville, MD, pp. 242.

US Pharmacopoeia 30/NF25, 2007c. US Pharmacopoeial Convention, Rockville, MD, pp. 385.

Vervaet, C., Remon, J.P., 1996. Influence of impeller design, method of screen perforation and perforation geometry on the quality of pellets made by extrusion/spheronisation. Int. J. Pharm. 133, 29-37.

Vertommen, J., Rombaut, P., Kinget, R., 1997. Shape and surface smoothness of pellets made in a rotary processor. Int. J. Pharm. 146, 21-29.

Wehrli, W., Knüsel, F., Schmid, K., Staehelin M., 1968. Interaction of rifamycin with bacterial RNA polymerase. Proc. Natl. Acad. Sci. 61, 667 73.

Whitehead, L., Fell, J.T., Collett, J.H., Sharma, H.L., Smith, A.M., 1998. Floating dosage forms: an in vivo study demonstrating prolonged gastric retention. J. Control. Rel. 55, 3–12.

WMA (World medical association), 2008. Declaration of Helsinki Ethical principles for medical research involving human subjects. 59th WMA General Assembly, Seoul.

Wlosnewski, J.C., Kumpugdee-Vollrath, M., Sriamornsak, P., 2009. Effect of drying technique and disintegrant on physical properties and drug release behaviour of microcrystalline cellulose-based pellets prepared by extrusion/spheronization. Chem. Eng. Res. Design. 88 (1), 100-108.

Xiaoqiang, X., Minjie, S., Feng, Z., Yiqiao, H., 2006. Floating matrix dosage form for phenoporlamine hydrochloride based on gas forming agent: In vitro and in vivo evaluation in healthy volunteers. Int. J. Pharm. 310, 139–145.

Xu, G., Groves, M.J., 2001. Effect of FITC-dextran molecular weight on its release from floating cetyl alcohol and HPMC tablets, J. Pharm. Pharmacol. 53 (1), 49– 56.

Yang, L., Eshraghi, J., Fassihi, R., 1999. A new intragastric delivery system for the treatment of Helicobacter pylori associated gastric ulcer: in vitro evaluation, J. Control. Release 57 (3), 215–222.

Chapter 3

Formulation development and evaluation of Isoniazid delayed release pellets

"Research is to see what everybody else has seen and to think what nobody else has thought"

..... Albert Szent-Gyorgyi

3

Formulation development and evaluation of Isoniazid delayed release pellets

3.1 Introduction

As reviewed earlier (section 1.6.5), literature survey revealed that, rifampicin interacts with isoniazid in the acidic medium of stomach to form inactive 3-formyl rifamycin isonicotinyl hydrazone (HYD). This is reflected in the poor bioavailability and also poor stability of rifampicin from the Anti TB - FDC formulation. Hence, there is a need to modify the FDC formulation in such a way that rifampicin and isoniazid are not released simultaneously in the stomach (Shishoo *et.al*., 2001; Singh *et.al*., 2001).

Isoniazid is poorly absorbed from the stomach because of its presence in the protonated form at acidic pH (pKa = 2). However, it is well absorbed from all the three segments of the intestine. Isoniazid is apparently much less permeated through the stomach and is mainly absorbed through the distal jejunum or ileum (almost 60%) (Marriapan and Singh, 2003). Accordingly, isoniazid should be formulated to target its release in intestine.

Oral ingestion is the predominant and most preferable route for drug delivery mainly due to their convenience of administration, patient compliance and their suitability for delivery of drugs for systemic effects. Following oral administration most drugs have to be absorbed into the blood to produce therapeutic action. However certain drugs have a “region-specific absorption” or ‘absorption window’. The region specific absoprtion may be due to various reasons, such as poor solubility at different pH values, poor stability in some GI regions, presence or absence of absorptive or efflux transporters, and presystemic metabolism in the gut wall (Kagan and Hoffman, 2008).

For region specific release of drugs *via* oral route of administration, multiparticulate drug delivery system, *viz.*, pellets, is the choice of drug delivery system. Pellets offer various advantages over single unit dosage form including minimal risk of dose dumping, flexibility of blending units with different release patterns, as well as short and reproducible gastric residence time (Kramer and Blume, 1994; Melia *et.al*., 1994).

However, the single most important factor responsible for the extensive use of pellets is the widespread use of controlled release technologies (Ghebre-Sellassi, 1989; Chatlapalli and Rohera, 1998). Due to their multiparticulate nature, pellets are mainly coated in order to either sustain drug release or to deliver a drug to the specific absorption site in the gastro-intestinal tract (e.g. enteric-coated or colon-targeted drug delivery). Delayed release pellets as dosage forms are especially suited for administration of drugs which are not stable in gastric fluids or can cause irritation of gastric mucosa and which are absorbed in the duodenum or upper intestine (Dukic´-Ott *et.al*., 2008). For delayed release formulation, aqueous / non-aqueous enteric polymeric dispersions are applied to solid oral dosage forms to facilitate targeted drug release to the intestine as a result of the pH-dependent solubility of the polymeric acidic functional groups. The most commonly used pH-sensitive enteric polymers today include cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate (HPMCP), and methacrylic acid copolymers. Enteric polymeric coatings play an important role in protecting drugs that are susceptible to acidic or enzymatic degradation in the stomach (Bruce *et.al*., 2003). After the acid-resistant coating has dissolved in the intestine, immediate drug release is essential in intestine for the absorption of the drug (Dukic´-Ott *et.al*., 2008). Extrusion-spheronization process is the most widely accepted method of pellet manufacturing (Ghebre-Sellassie and Knoch, 2007).

3.2 Proposed formulation design of anti-TB FDC

The proposed formulation was designed to release the two drugs in a controlled manner with rifampicin being released in the stomach and isoniazid 3-4 h later in the intestine. The proposed formulation was designed to incorporate the following components of anti-TB FDC in a capsule:

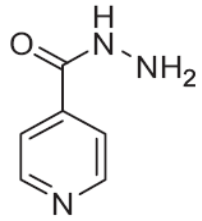
- **Rifampicin:** Total dose of rifampicin was subdivided into two components-
  - (i) Immediate release pellets of rifampicin- loading dose of rifampicin
  - (ii) Gastroretentive floating pellets of rifampicin- maintenance dose of rifampicin
- **Isoniazid:** Delayed release pellets of isoniazid

In this chapter, formulation development and evaluation of delayed release isoniazid pellets will be discussed.

3.3. Drug profile- Isoniazid

- Molecular formula $C_6H_7N_3O$

Chemical structure



- Generic name: isonicotinic acid hydrazide; isonicotinoylhydrazine; isonicotinylhydrazine
- Molecular weight: 137.14
- Solubility: soluble in water (~14% at 25ºC, ~26% at 40ºC) ethanol: (~2% at 25ºC), boiling ethanol (~10%), chloroform (~0.1%). Practically insoluble in ether, benzene.
- Polarity (Log P): 0.64
- Acidity/basicity: pH of a 1% aqueous solution 5.5 to 6.5
- Stability: Very stable in DMSO; rather stable in water
- Melting point: 171.4ºC
- Optimal human dosage: 5 mg/kg for adults, 10-20 mg/kg for children. Adult dosing generally 300 mg capsule administered orally, once daily; or 15 mg/kg up to 900 mg/day, two or three times/week, ideally dose administered 1 h before or 2 h after a meal. Concomitant administration of pyridoxine (B_6) recommended for malnourished patients, adolescents, and those predisposed to neuropathy (e.g. diabetic).Can also be given intramuscularly or intravenously (WHO, 2003).
- *In-vitro* potency: For *M. tuberculosis* H37Rv, MIC is 0.025 mg/ml.

Mechanism of action

Isoniazid is a prodrug activated by catalase-peroxidase hemoprotein, KatG. Isoniazid inhibits InhA, a nicotinamide adenine dinucleotide (NADH)-specific enoyl-acyl carrier protein (ACP) reductase involved in fatty acid synthesis (Petri, 2001).

Spectrum of activity

Isoniazid is a bactericidal agent active against organisms of the genus *Mycobacterium*, specifically *M. tuberculosis*, *M. bovis* and *M. kansasii*. Isoniazid is bactericidal to rapidly-dividing mycobacteria, but is bacteristatic if the mycobacterium is slow-growing. Isoniazid is highly specific, being active against only a subset of the mycobacteria and largely ineffective against other microorganisms; this is in part due to several unusual aspects of metabolism, exemplified in *M. tuberculosis*, including unusually high KatG activity and a defective drug efflux mechanism (Zang, 2003).

Pharmacokinetics of Isoniazid

Absorption

Isoniazid is readily absorbed when administered either orally or parentrally. Peak plasma concentrations of 3-8 μg/ml develop 1-2 h after fasting dose of 300 mg orally. Aluminium containing antacids may interfere with the absorption of isoniazid (Hurwitz and Scholzman, 1974; Becker *et.al*., 2007).

Distribution

Isoniazid diffuses readily into all body fluids and cells. Isoniazid is not considered to be bound appreciably to plasma proteins. The concentration of the drug is initially higher in the plasma and muscle than in the infected tissue, but the latter retains the drug for long time in quantities well above those required for bacteriostasis (Holdiness, 1984; Becker, *et.al*., 2007).

Metabolism

The plasma half-life of isoniazid ranges from 1-4 h, those who are fast acetylators because of genetic variations, having short half-lives. The primary metabolic route is acetylation of isoniazid to acetylisoniazid by N-acetyltransferase, form in the liver and small intestine. Acetylisoniazid is then hydrolysed to isonicotinic acid and monacetylhydrazine, isonicotinic acid is conjugated with glycine to isonicotinyl glycine and monoacetylhydrazine is further acetylated to diacetylhydrazine. Some unmetabolized isoniazid is conjugated to hydrazones. The metabolites of isoniazid have no tuberculostatic activity and are non-toxic (Becker *et.al*., 2007).

Excretion

Elimination of isoniazid from the body is dependent upon its genetically controlled rate of acetylation. Excretion is primarily renal. From 75% to 95% of dose of isoniazid is excreted in the urine within 24 h, mostly as metabolites. In patients with normal renal function, over 70% of a dose appears in the urine in 24 h. Of this amount, 93% of the isoniazid excreted in urine in fast acetyltors in the form of N-acetylisoniazid and 63% in slow acetylators as N-acetylisoniazid (Gilbaldi, 1984). Small amounts of drug are also excreted in faeces (Becker *et.al*., 2007).

Drug-drug interactions

Isoniazid interacts with the cytochrome P-450 system, especially CYP2E1, where it shows a biphasic inhibition induction; it causes increases in serum concentrations of various drugs, especially phenytoin and carbamazepine, increases the effects of warfarin and theophylline, inhibits metabolism of benzodiazepines, and inhibits monoamine oxidase and histaminases. Isoniazid should not be administered with food, as studies have shown that this significantly reduces its bioavailability (Petri, 2001).

Adverse effects of isoniazid

Central Nervous System (CNS) effects: Peripheral neuropathy is the most common CNS-related toxic effect. It is dose-related, occurs most often in the malnourished and in those predisposed to neuritis (e.g., alcoholics and diabetics), and is usually preceded by paraesthesias of the feet and hands. The incidence is higher in "slow acetylators". Other neurotoxic effects, which are uncommon with conventional doses, are convulsions, toxic encephalopathy, optic neuritis and atrophy, memory impairment and toxic psychosis.

Hepatic effects: Isoniazid does carry a specific warning of the potential for liver toxicity. Liver toxicity and hepatitis risks are increased with concomitant use of carbamazepine, phenobarbital, rifampicin, and alcohol abuse. Elevated serum transaminase (SGOT SGPT), bilirubinaemia, bilirubinuria, jaundice, and occasionally severe and sometimes fatal hepatitiscan occur with normal dosing regimens. The common prodromal symptoms of hepatitis are anorexia nausea, vomiting, fatigue, malaise, and weakness. Mild hepatic dysfunction, evidenced by mild and transient elevation of serum transaminase levels appears in the first 1-3M of treatment but can occur at any time during therapy. In most instances enzyme levels return to normal, and generally there is

no necessity to discontinue medication during the period of mild serum transaminase elevation. The frequency of progressive liver damage increases with age. It is rare in persons under 20, but occurs in up to 2.3% of those over 50 years of age (Petri, 2001; Zang, 2003).

Gastrointestinal effects: Nausea, vomiting, epigastric distress and dark urine can occur but are rare. Haematological effects: agranulocytosis; hemolytic, sideroblastic, or aplastic anaemia, thrombocytopenia; and eosinophilia can occur (Petri, 2001).

Endocrine and metabolic: Pyridoxine deficiency, pellagra, hyperglycaemia, acidosis and gynecomastia can occur (Zang, 2003).

Hypersensitivity: Fever, skin rashes, lymphadenopathy and vasculitis can occur (Jayaram *et.al*., 2004).

Indications

The primary indications for isoniazid is for the treatment of tuberculosis (pulmonary and extrapulmonary lesions (Becker *et.al*., 2007).

Contraindications

Isoniazid is contraindicated in known cases of hypersensitivity to the drug. It is contraindicated in alcoholics with severely impaired liver function and with jaundice (Zang, 2003).

3.4 Formulation development and evaluation of delayed release multiparticulate system of isoniazid

3.4.1 Materials

Isoniazid was obtained as a gift sample from Cadila Pharmaceuticals Limited, Ahmedabad. The excipients and chemicals used in the formulation development and its evaluation like Avicel PH101, PVP 90, Acryl eze, Cellulose acetate phthalate, Eudragit RL100 etc are enlisted in Annexure 1.

3.4.2 Methods

3.4.2.1 Preparation of core isoniazid pellets

3.4.2.1.1 Granulation

Isoniazid was blended with Avicel PH 101. The batch size was 250 g of dry material and the isoniazid load varied from 55 to 85% (w/w). The powders were dry mixed for 5 min. at 60 rpm in a planetary mixer (Kalweka, India). The mixture was wetted with purified water (40 - 43% of the total mass) and polyvinyl pyrollidone and granulated for 5 min. using the same equipment and mixing speed.

3.4.2.1.2 Extrusion

The wet mass was extruded at an extrusion speed of 150 rpm by means of a gravity fed extruder (R.R Enterprise, Mumbai).

3.4.2.1.3 Spheronization

The extrudates were spheronized (at 800 rpm during 8 min.) in a spheronizer (R. R. Enterprise, Mumbai) using a friction plate with cross-hatched geometry.

3.4.2.1.4 Drying

The pellets were dried in a fluidised bed dryer (Niro Aeromatic, Switzerland) at 50°C for 10 min.

3.4.2.2 Coating of core isoniazid pellets

Modal fraction of beads from the optimized batch was subjected to delayed release coating using various polymers like, Cellulose acetate phthalate, Acryl-eze ®, Eudragit RL- 100, etc. Aqueous coating on the isoniazid core pellets was carried out in fluidized bed coater.

The process conditions were 'pre-warming of the cores at 40°C for 10 min.; spray nozzle diameter, 1 mm; atomizing air pressure, 1 bar; air flow rate, 80 m^3 h^{-1}; inlet air temperature, 40°C; product temperature 32-35°C; spray rate, 1.5 ml $min.^{-1}$; post drying at 40°C for 10 min.'. The core isoniazid pellets were coated upto 35% weight gain.

3.4.3 Optimization of core isoniazid pellets using response surface methodology

A 2^3 FFD was used for optimizing the formulation. The studied factors were: the amount of granulating fluid; purified water (X_1, % w/w of dry blend) and amount of binder; Kollidon® 90 (X_2, % w/w of dry blend) and the spheronization speed (X_3, revolutions per minute, rpm). The responses studied were Usable yield (Y_1, %theoretical,), Pellet size (Y_2, µm), Pellips (Y_3), Porosity (Y_4, %), Abrasion resistance (Y_5, %), Mechanical crushing force (Y_6, Newtons), Residual moisture (Y_7, %) and dissolution efficiency (DE) at 15 min. (Y_8, %). These studied factors along with their levels and the corresponding responses are summarized in Table 19 and experimental formulations are listed in Table 20.

Table 19. 2^3 Full Factorial experimental design: Factors and Responses

Factors	Levels of the factors	
	-1	+1
X_1 = Amount of granulating fluid, water	35% w/w of dry mix	55% w/w of dry mix
X_2 = Amount of binder, Kollidon® 90	0% w/w of dry mix	3% w/w of dry mix
X_3 = Spheronization speed	700 rpm	1000 rpm
Responses		
Y_1= Usable yield (% theoretical)	Y_5 = Abrasion resistance (%)	
Y_2= Pellet size (µm)	Y_6= Mechanical crushing force (Newton)	
Y_3= Pellips	Y_7= Residual moisture (%)	
Y_4= Porosity (%)	Y_8= Dissolution Efficiency; DE (%)	

Table 20. Formulation design as per the 2^3 full factorial experimental design

Formulation run	X_1	X_2	X_3
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1

3.4.3.1 Statistical analysis of the data and validation of the optimization model

The design expert version 7.1 software (Minnesota, USA) was used in the current study for the generation and evaluation of statistical experimental design. Polynomial models including interaction terms were generated for all the response variables using multiple linear regression analysis. Graphical representation of the influence of factors and their interaction were generated.

In order to validate the polynomial equations, one optimum checkpoint (formulation composition and process) and two random checkpoints were selected by intensive grid search, performed over the entire experimental domain. The criterion for selection of optimum check point was mainly based on the highest possible values of response parameters, i.e. usable yield, porosity, mechanical crushing force, DE and pellips; while lowest possible values of responses, namely, size, abrasion resistance and water content. Formulations corresponding to these three check points were prepared and evaluated for all the eight responses (Y_1 to Y_8). The resultant experimental data of response properties were subsequently compared quantitatively with the predicted values.

3.5 Evaluation of isoniazid pellets

3.5.1 Particle size distribution

Pellets size distribution (span) was carried out by Malvern Mastersizer (Malvern 2000, Malvern Instruments, UK). All the measurements were carried out in triplicate. 50^{th} percentile diameter of the cumulative particle size distribution was considered as mean pellet size (Koo and Heng, 2001).

3.5.2 Usable yield (% theoretical)

The usable yield of the pellets was determined by sieve analysis, using a sieve shaker (EMS-8, Electrolab, India) equipped with (600-2360 µm) sieves for 5 min. at an amplitude of 2 mm. The pellet yield was calculated based on the pellet fraction between #14/22 and presented as the percent of the total pellet weight (Howard *et.al*., 2006). This size fraction was used for all further measurements.

3.5.3 Sphericity and shape analysis

The spherecity and shape of the pellets were determined using an image analysis system. Photomicrographs of pellets were taken with a digital camera linked with a stereomicroscope system a stereomicroscope Leica S4E (Germany). The captured images were analysed by image analysis software (AnalySIS, Soft Imaging System, v. 5.2, Münster, Germany). Around 50 pellets were analysed from every batch. Shape of each individual pellet was characterised as pellips (Podczeck *et.al*., 1999; Koo and Heng 2001; Almeida-Prieto *et.al*., 2007)

$$Pellips = \frac{P}{\pi \, x \, d_{max}} \qquad \text{--(15)}$$

Where, P is the perimeter and d_{max} is maximum diameter of the pellet, calculated directly by using image analysis software.

3.5.4 Surface characterization

Morphological examination of the surface was carried out using a SEM. SEM of pellets was obtained using JEOL JSM 6100 (JEOL, Japan). The particles were vacuum dried, coated with thin gold-palladium layer by sputter coater unit (JEOL JFM 1100) and observed microscopically at an accelerating voltage of 5.0 kV.

3.5.5 Abrasion resistance

This test was carried out only on uncoated beads. The friability of the uncoated pellets (#14/ 22 fraction) was determined. Weighed amount pellets were subjected to test along with 24 steel balls (diameter about 2 mm) in a Roche friabilator for 100 revolutions at 25 rpm and then sieved through a #22 sieve. The percent weight loss was then calculated (Howard *et.al*., 2006). Each batch was analysed in triplicate.

3.5.6 Mechanical crushing force

At least 20 pellets from the Modal size fraction of each formulation were evaluated for their diametral crushing force using a tablet strength tester (EH 01, Electrolab, India) (Sousa *et.al*., 2002; Newton *et.al*., 2007).

3.5.7 Densities and Angle of repose

The bulk density was determined by pouring weighed amount of pellets into a graduated glass cylinder. The bulk density was calculated by dividing the weight by the occupied volume. The tapped density was determined using a tapped density tester in which the glass cylinder was tapped 1000 times (750 taps followed by 250 taps) (USP, 2007a). All measurements were carried out in triplicate. Angle of repose was determined using reposograph.

3.5.8 Porosity

Pellet porosity was calculated using the following equation, for percent effective porosity (Chopra *et.al*., 2001; Steckel and Mindermann-Nogly, 2004).

$$\%\varepsilon = [(\rho t - \rho b) / \rho b] \times 100 \qquad \text{--(16)}$$

Where ε = effective porosity, ρt = true density and ρb = bulk density. The true density of the powder formulation was determined in triplicate using Helium pycnometry (Smart Pycno 30, Smart Instruments, Mumbai).

3.5.9 Residual moisture content

The residual water content present in the pellets after drying was determined by using Karl Fischer titrator (Systronics Universal titrator 353, India), USP Method I. The equipment was pre-calibrated and standardised with di-sodium tartrate. Pellets, approximately 250 mg, were accurately weighed and immediately placed in the moisture analyser for titration with Karl Fischer reagent. Each batch was analysed in triplicate. (USP, 2007b).

3.5.10 Drug content

The isoniazid pellets both, uncoated beads and coated beads were assayed as per US pharmacopoeial method, using HPLC. The analysis was carried out on C_{18} column with 95% phosphate buffer (pH 6.9) and 5% methanol as mobile phase at a flow rate 1ml/min. and detection wavelength 263 nm (USP, 2007c).

3.5.10.1 Gastric acid resistance test

Acid resistance test is a significant index of drug dissolution performance of enteric coated formulations. Modal fraction of coated beads was subjected for acid resistance test in USP dissolution test apparatus –I (SR-8, Hanson Research, Chatsworth, USA). Weighed amount of beads were placed in the basket and test was carried out in 0.1N HCl for 2h at 100rpm. Isoniazid released at 1h and 2h, in 0.1 N HCl was estimated as per method specified in USP (USP, 2007d). Minimal amount of drug release in this test is indicative of gastric acid resistance.

3.5.10.2 Drug release

Release studies were carried out for both uncoated as well as coated beads. For carrying out release rate study, USP method B, for delayed release formulations, was followed. Isoniazid pellets equivalent to 300 mg were subjected for dissolution test studies. The test was carried out in acidic media (0.1N HCl) for 2h followed by 45 min. in phosphate buffer pH 6.8. The test was carried out in at 100 rpm in USP dissolution test USP test apparatus- II with 900ml of media. The samples (5ml) were withdrawn and replaced with an equal amount of fresh media at 60 min., 120 min. (in acidic media) followed by sampling at 135 min., 150 min., 165 min., and 180 min. (in phosphate buffer pH 6.8). Isoniazid released in 0.1 N HCl was estimated as per method specified in USP (USP 2007; Joshi *et.al*., 2008) and isoniazid released in pH 6.8 phosphate buffer was measured at λ_{max} 263 nm by a validated spectrophotometric method (Rastogi *et.al*., 2007).

3.6 Stability studies

The isoniazid delayed release pellets prepared were subjected for stability studies. For this part of the study, the pellets were filled into empty hard gelatine capsule shells and were stored in tightly closed HDPE containers and in aluminium pack. The stability studies were carried out at room temperature and accelerated relative humidity conditions as per ICH guidelines (ICH, 2003). The accelerated stability conditions were 40°C ± 2°C/75% RH ± 5%. The stability samples were analysed at 1, 2, 3 and 6 M. The

assay, water content and dissolution studies of these pellets were carried as per the methods described earlier. To ensure the equivalence in release profile of the stability samples with that of initial samples, the fit factor, f_2, was calculated. The formula used for calculating f_2 values is shown in Eq. (17):

$$f_2 = 50 \log\{[1+ 1/n\sum_{t=1}^{n}(R_t-T_t)]^{0.5} \text{ X } 100\} \quad \text{--(17)}$$

3.7 Results and Discussion

Ideally, a high percentage of pellets should be produced within a desired size range from a successful extrusion-spheronization process. Also, it is important to determine the pellet size, size distribution, shape, abrasion resistance and mechanical strength as these parameters determine the quality of pellets produced.

In the present study, experimental design methodology was exploited systematically for evaluating the effect of varying the amount of granulating fluid; water, binder; Kollidon® 90, and spheronization speed as well as to highlight any interaction among the components on the micromeritic, mechanical and release properties of isoniazid pellets. This will facilitate the identification of the most significant factors influencing these properties and establishing their best levels for optimizing the considered experimental responses.

Mathematical relationship was generated between the factors and responses for determining the levels of factors, which yield optimum responses. A second order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad \text{-- (18)}$$

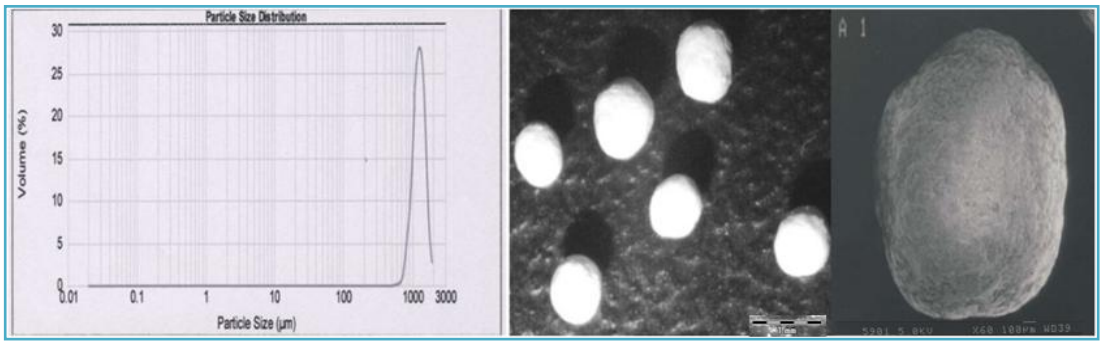
Where, b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of eight experimental runs; b_1 to b_3 are the coefficients computed from the observed experimental values of Y; and X_1, X_2 and X_3 are the coded levels of factors. The terms XiXj (i and j = 1, 2 and 3) represent the interaction terms. The equation represents the quantitative effect of factors (X_1, X_2 and X_3) upon the each of the responses; Y_1 to Y_8. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. A positive sign in front of the terms indicates synergistic effect while

negative sign indicates antagonistic effect of the factors. ANOVA was applied for estimating the significance of the model, at 5% significance level. A model is considered significant if the *p*-value is less than 0.05.

3.7.1 Pellet yield and size distribution

For a successful extrusion-spheronization process and the formulation, a high percentage of pellets should be produced within a desired size range. Pellets prepared by the process of extrusion and spheronization generally have a mean size between 0.5 and 1.5 mm depending on the diameter of the hole in the extruder die plate. Size polydispersity of pellets is an important factor to be considered if the pellets are to be coated for modifying the release of the drug. The uniformity of the coating requires narrow and homogeneous pellet size distribution. A narrow size distribution will ensure minimum variation in coating thickness throughout the batch of pellets and therefore result in a uniform performance of pellets within the batch (Mehta, 1989). Span is the indicator of size polydispersity. Core pellets produced were found to have mean particle size of 1.43 mm and span value of 0.418. Low span value of core pellets indicates narrow size distribution and was found to follow Gaussian distribution (Fig 22).

Fig 22. Particle size distribution (by Malvern), Shape characterization (by Image analysis) and Surface characterization (by SEM) respectively, of Uncoated isoniazid pellets



For usable yield, amount of granulating fluid (X_1) and amount of binder (X_2) and their interaction terms (X_1X_2) were found to be significant while for size distribution, amount of granulating fluid (X_1) and amount of binder (X_2) were found to be significant ($p<0.05$) (Table 21). Therefore, when evaluating the influence of a specific factor (variable) on process yield significant interactions between amount of granulating fluid and binder level should be considered. The predicted R-squared value was in reasonable agreement with the R-squared value adjusted for the degrees of freedom. The regression

equation for usable yield (Y_1) and pellet size distribution (Y_2) and in terms of the coded values is the following Eq.

$$Y_1 = 86.65 - 3.075X_1 - 3.925X_2 + 0.050X_3 + 1.45\ X_1X_2 + 0.675X_1X_3 - 0.375X_2X_3 \quad \text{--(19)}$$

$$Y_2 = 966.951 + 85.826X_1 + 103.786X_2 + 8.201X_3 - 48.599\ X_1X_2 - 17.669X_1X_3 + 6.691X_2X_3 \quad \text{--(20)}$$

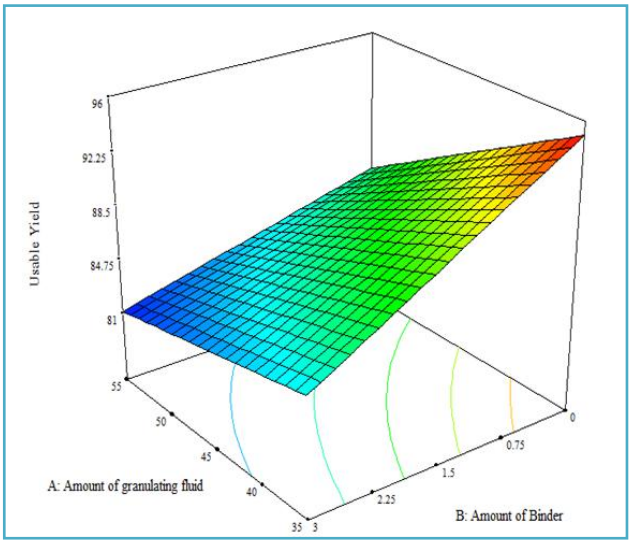
Results indicated that increasing the concentration of binder and amount of granulating fluid, increases the mean pellet size but decreases the yield in the desirable size range; 700-1190 µm. The influence of amount of granulating fluid and amount of binder at fixed spheronization sped on usable yield is shown graphically in Fig 23. Graphical analysis shows that usable yield varies in a nearly linear descending pattern with the change in amount of binder and granulating fluid. Usable yield appears to be inversely influenced by amount of binder as well as amount of granulating fluid (Eq. 19 and Fig 23). It can be clearly concluded from the Fig 23, that effect of amount of binder seems to be more pronounced as compared with that of granulating fluid.

Table 21. ANOVA results for usable yield and size distribution

Usable Yield		**Size distribution**	
Source	*p* value	Source	*p* value
Model	**0.0435**	**Model**	**0.0300**
X_1	**0.0310**	X_1	**0.0171**
X_2	**0.0243**	X_2	**0.0142**
X_3	0.7952	X_3	0.1749
$X_1\ X_2$	**0.0656**	$X_1\ X_2$	**0.0303**
$X_1\ X_3$	0.1392	$X_1\ X_3$	0.0828
$X_2\ X_3$	0.2422	$X_2\ X_3$	0.2117
R^2	0.9992	**R^2**	0.9997
Adjusted R^2	0.9943	**Adjusted R^2**	0.9982

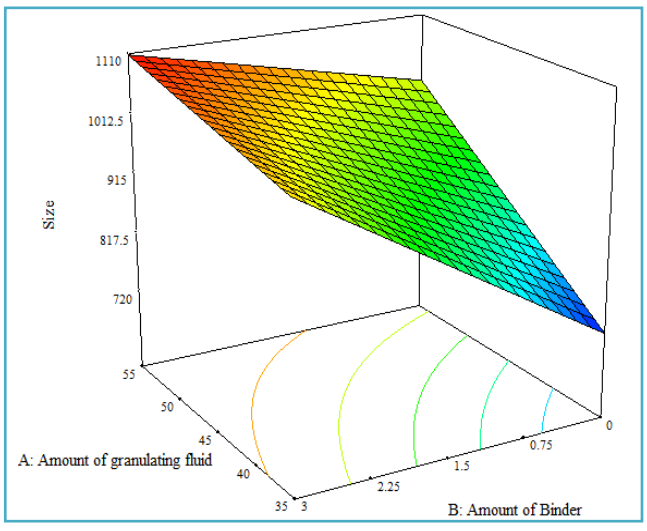
Significant terms are shown in bold type

Fig 23. Response surface plot showing the influence of amount of granulating fluid and amount of binder at fixed spheronization sped on usable yield



The influence of amount of granulating fluid and amount of binder with fixed spheronization speed on size is shown in the response surface plot, Fig 24. This figure depicts that pellet size exhibits a near linear trend but in a descending manner. It was found that increasing the concentration of binder, increases the mean pellet size but decreases the yield in the desirable size range; 700-1190 µm. This may be because of higher cohesiveness and inter-particulate adherence provided to the wet mass by binder and granulating fluid, resulting in pellet agglomeration. This agglomeration increases the quantity of abnormally large particles thereby, decreasing the usable yield.

Fig 24. Response surface plot showing the influence of amount of granulating fluid and amount of binder with fixed spheronization speed on size



3.7.2 Shape analysis

If the process of extrusion and spheronization is not optimized, pellet shapes can vary ranging from rounded cylinders to dumbbells and ellipsoidal. While it is desirable to obtain high usable yield of durable pellets, it is ultimately the shape of the collected material that is critical for a number of processing advantages (e.g. a free flowing and uniformly coated product). It is eventually the work of spheronization process, to fragment the extrudate through interactions with the frictional plate, and subsequently smoothen the fragments into the spherical pellets. In literature, a variety of parameters have been used to express the shape of the pellets like Aspect ratio, Roundness score, Circularity, Pellips, Elongation, Projection sphericity etc (Podczeck *et.al.*, 1999; Steckel and Mindermann-Nogly, 2004; Howard *et.al.*, 2006; Almeida-Prieto *et.al.*, 2007).

The mathematical relationship that describes the influence of amount of granulating fluid, amount of binder and spheronization speed on pellips is shown in Eq. 21.

$$Y_3 = 0.909 + 0.009\ X_1 + 0.0006\ X_2 + 0.031X_3 - 0.002X_1X_2 - 0.002X_1X_3 - 0.003X_2X_3 \quad \text{---(21)}$$

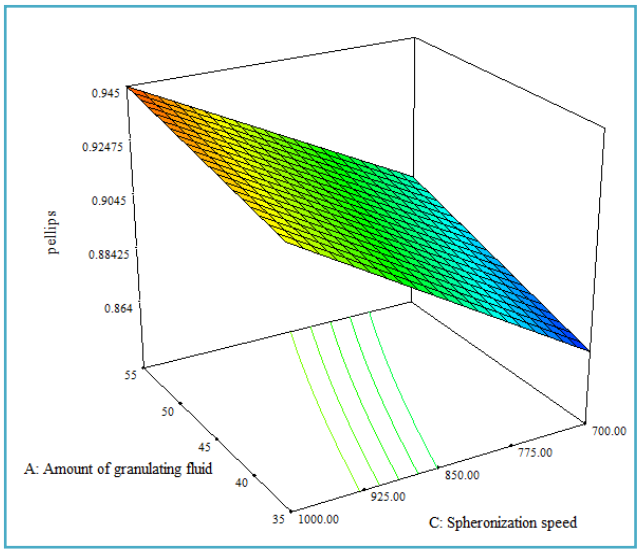
Graphical representation of the influence of amount of granulating fluid and spheronization speed with fixed amount of binder on pellips is shown in Fig 25. It can be concluded from the statistical and graphical analysis that pellips is significantly affected by the amount of granulating fluid; water and the spheronization speed in a linear fashion (Table 22). However, the effect of spheronization speed is more pronounced as compared to that of amount of granulating fluid. This can ascribed to the fact that rounding of pellets in the spheronizer is a function of plasticity of the extrudates, where water acts as a plasticizer. (Lustig-Gustafsson *et.al.*, 1999). At higher speed of spheronization, there is a rapid availability of water, which acts as a plasticizer, at the initial stage of the process of spheronization. Thus, forming more spherical pellets in this process.

Table 22. ANOVA results for shape analysis-pellips

Pellips	
Source	*p* value
Model	**0.000263**
X_1	**0.0261**
X_2	0.344
X_3	**0.00779**
X_1 X_2	0.1000
X_1 X_3	0.1000
X_2 X_3	0.0700
R^2	0.999
Adjusted R^2	0.999

Significant terms are shown in bold type

Fig 25. Response surface plot showing the influence of amount of granulating fluid and spheronization speed with fixed amount of binder on pellips



3.7.3 Pellet porosity

Pellet porosity, a vital characteristic, strongly depends on composition of pellet, volume of the wetting liquid, spheronization and drying conditions. This will critically determine the relevant properties such as friability, flowability, wettability, adhesion to various substrates and drug release profile in different ways. The porosity of the pellets was determined, since a smooth pellet surface structure is important for a successful coating process as well as affects the release profile of the drug. This also has the potential to

change the ability of a film to adhere to the surface of the pellets (Go´mez-Carracedo *et.al*., 2009). Influence of amount of granulating fluid, amount of binder and spheronization speed on porosity has been mathematically represented in Eq. 22.

$$Y_4 = 44.94 + 0.42X_1 - 0.41\ X_2 - 0.39X_3 + 0.70\ X_1X_2 + 0.95X_1X_3 + 1.85X_2X_3 \quad ..(22)$$

The results of ANOVA for pellets porosity are enlisted in Table 23. It was found that for porosity, all the variables i.e. amount of granulating fluid (X_1) , amount of binder (X_2), spheronization speed (X_3)and their interaction terms (X_1X_2, X_1X_3, X_2X_3) were found to be significant ($p<0.05$) (Table 23). The predicted R-squared value was in reasonable agreement with the R-squared value adjusted for the degrees of freedom.

Table 23. ANOVA results for porosity

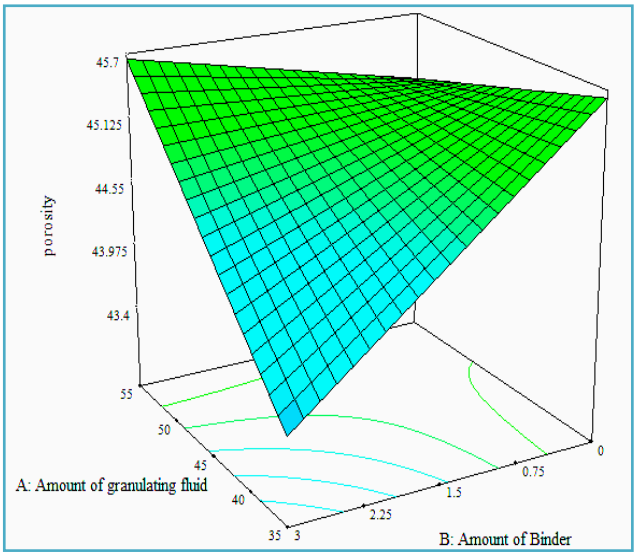
Porosity	
Source	*p* value
Model	**0.0196**
X_1	**0.0356**
X_2	**0.0365**
X_3	**0.0391**
$X_1\ X_2$	**0.0216**
$X_1\ X_3$	**0.0159**
$X_2\ X_3$	**0.0084**
R^2	0.9999
Adjusted R^2	0.9992

Significant terms are shown in bold type

The influence of the amount of granulating fluid and amount of binder on porosity is graphical represented in Fig 26. There appears to be significant negative influence of individual components i.e. amount of granulating fluid, amount of binder and spheronization speed on the porosity of the isoniazid pellets as depicted in the graph Fig 26 and Eq. 22 (*p*-value<0.05, Table 23). This can be explained by the fact that during spheronization of extrudates, water migrates to the surface resulting in reduction of voids, which in turn, leads to further densification and reduced porosity. It can be clearly seen from the graph that, a ‘region of maxima’ is found between lower level of binder and higher level of granulating fluid (Fig 26). This can be attributed to the fact that, as the amount of binder is increased, porosity decreases. This may be attributed to the fact

that increased amount of binder leads to formation of dense and cohesive mass, with reduced porosity. While, a direct linear relation was found between the amount of granulating fluid and porosity i.e. an increase in water content leads to increase in porosity. This may be due to the fact that in wet pellets water occupies a majority of the surface area of the pellet, which gets evaporated during drying process, creating void spaces in the pellet structure and thus forming a porous pellet structure.

Fig 25. Response surface plot showing the influence of amount of granulating fluid and amount of binder on porosity



3.7.4 Friability and mechanical crushing force

Abrasion resistance is designed to assess the resistance of the pellet surface to abrasion, which pellets will encounter during further processing and shipping, whereas, mechanical crushing force gives indication of its mechanical robustness.

Pellets with high resistance to abrasion are desirable, as they are likely to retain their integrity on handling and during further processing, such as coating. The mathematical relationship between amount of granulating fluid, amount of binder and spheronization speed for friability is shown in Eq. 23.

$$Y_5 = 0.862 - 0.137X_1 - 0.737\ X_2 - 0.088X_3 + 0.062\ X_1X_2 + 0.013X_1X_3 - 0.062X_2X_3 \quad \text{--(23)}$$

The amount of binder was found to have significant influence on abrasion resistance (p<0.05) as shown in Table 24. The response surface plot for the influence of amount of granulating fluid and amount of binder at fixed spheronization speed on friability is shown in Fig 27a. Graphical analysis reveals that amount of binder and amount of

granulating fluid affects friability in almost linear fashion. However, amount of binder seems to be more pronounced and is inversly affecting the friability (Fig 27a). This implies that in order to minimize the abrasion resistance, amount of binder needs to be maximized.

Mathematical relationship between amount of granulating fluid, amount of binder and spheronization speed for mechanical strength is described in Eq. 24.

$$Y_6 = 6.9 + 0.175X_1 + 1.575\ X_2 - 0.200X_3 - 0.050\ X_1X_2 - 0.075X_1X_3 + 0.125X_2X_3 \quad --(24)$$

For mechanical strength of individual pellet, values for all the eight batches are comparable and contribution of each factor and their interaction was found to be statistically non-significant ($p>0.05$, Table 24). This might be due to the fact that strength measurements were carried out on pellets of same size fraction, 0.8–1.0 mm. The response surface plot for the influence of amount of granulating fluid and amount of binder at fixed spheronization speed on mechanical strength is shown in Fig 26b. Nevertheless, graphical analysis reveals that amount of binder inversely affects the mechanical strength of the pellet in almost a linear fashion (Fig 27b). However, the effect of amount of binder seems to have a more pronounced effect as compared to amount of granulating fluid. Pellets lacking sufficient binding property at the surface will experience greater damage during attrition and make them more vulnerable to wear and tear.

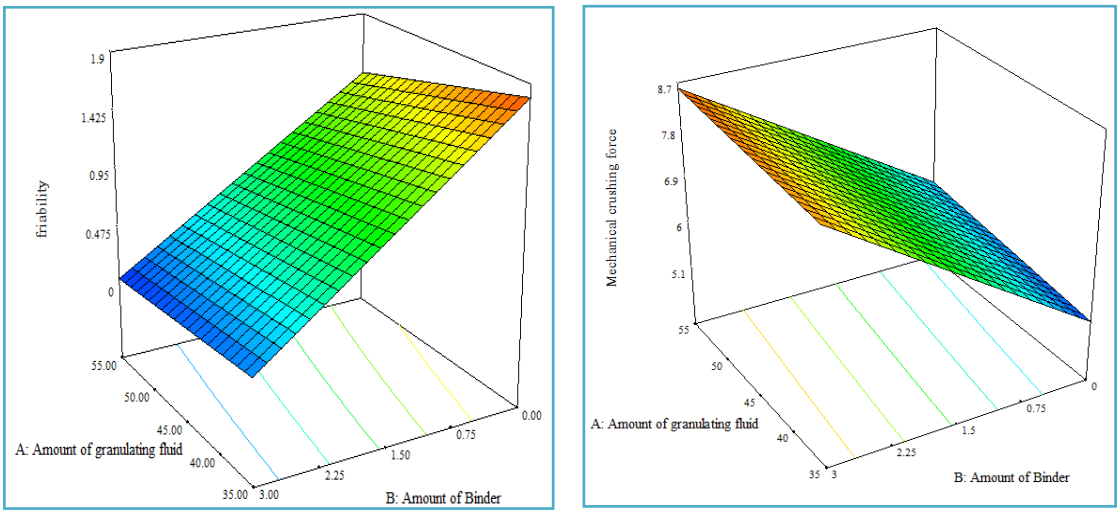
Table 24. ANOVA results for friability and mechanical crushing force

Abrasion resistance		**Mechanical strength**	
Source	*p* value	Source	*p* value
Model	**0.00922**	**Model**	**0.0173**
X_1	0.1695	X_1	0.4511
X_2	**0.0323**	X_2	0.0604
X_3	0.2578	X_3	0.4097
$X_1\ X_2$	0.3440	$X_1\ X_2$	0.7952
$X_1\ X_3$	0.7952	$X_1\ X_3$	0.7048
$X_2\ X_3$	0.3400	$X_2\ X_3$	0.5577
R^2	0.9979	**R^2**	0.9913
Adjusted R^2	0.9830	**Adjusted R^2**	0.9913

Significant terms are shown in bold type

Fig 27. Response surface plot showing the influence of amount of granulating fluid and amount of binder at fixed spheronization speed on **(a)** Friability, **(b)** Mechanical strength

(a). (b).



3.7.5 Residual moisture

Mathematical relationship between amount of granulating fluid, amount of binder and spheronization speed for moisture content is described in Eq. 24. ANOVA for residual moisture is shown in Table 25.

Only amount of granulating fluid was found to have significant effect on the residual moisture (Table 25). None of the cross-product term was found to be significant.

$$Y_7 = 1.815-0.027X_1+0.019X_2 -0.042X_3 +0.021\ X_1X_2 -0.005X_1X_3 +0.003X_2X_3 \quad --(25)$$

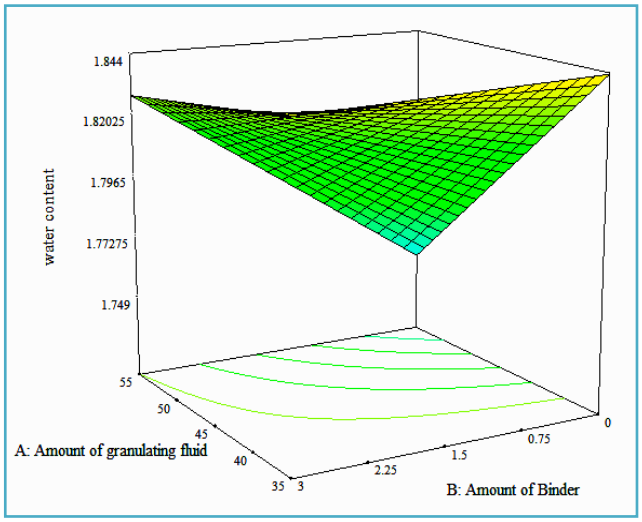
The response surface plot for the influence of amount of granulating fluid and amount of binder at fixed spheronization speed on moisture content is shown in Fig 28.Graphical analysis and equation reveals that effect of granulating fluid seems to have minor and linear effect on moisture content (Fig 28). This may be due to the fact that for the standard drying conditions, it was found that, the differences in the residual moisture of the pellets was very small (statistically non significant), indicating that in spite of the different initial water contents of the pellets, the drying process efficiently removed the free water added during the initial wet massing stage.

Table 25. ANOVA results for moisture content

Moisture content	
Source	*p* value
Model	**0.01414**
X_1	**0.01037**
X_2	0.1450
X_3	0.0655
X_1 X_2	0.1331
X_1 X_3	0.4823
X_2 X_3	0.5817
R^2	0.9943
Adjusted R^2	0.9959

Significant terms are shown in bold

Fig 28. Response surface plot showing the influence of amount of granulating fluid and amount of binder on moisture content



3.7.6 Drug release

Dissolution efficiency (DE) is a model-independent parameter widely employed as a significant index of drug release and dissolution performance (Costa and Lobo, 2001; Menegola *et.al*., 2007). In the present study, all the formulation batches showed statistically non-significant and comparable DE. ANOVA for DE is tabulated in Table 26. *p*–values of each coefficient indicate non-significant effect of the individual factors or their interactions on the response (Table 26). Mathematical relationship between amount of granulating fluid, amount of binder and spheronization speed for DE is

described in Eq. 26. This behaviour can be attributed to the highly soluble nature of isoniazid (~125mg ml^{-1}), which is a borderline of Class I and Class III of Biopharmaceutical Classification System (BCS) (Becker *et.al*., 2007).

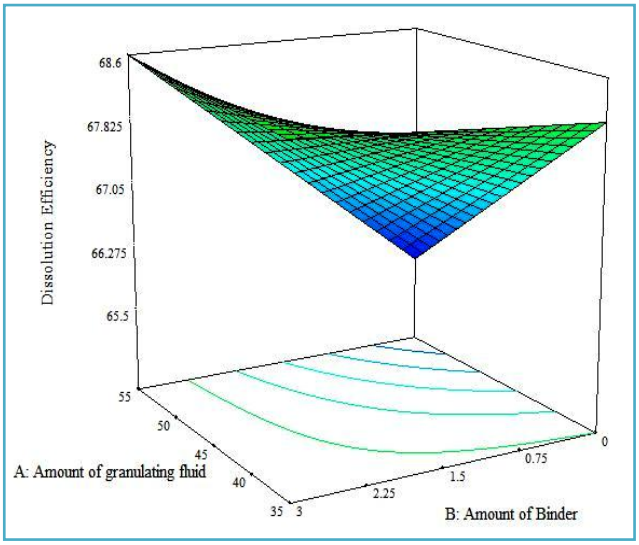
$$Y_8 = 67.65-0.575X_1+0.825X_2 -0.200X_3 +0.700\ X_1X_2 -1.575X_1X_3 -1.3753X_2X_3 \text{ --(26)}$$

Table 26. ANOVA results for DE

DE	
Source	*p* value
Model	**0.01520**
X_1	0.1625
X_2	0.1145
X_3	0.4097
X_1 X_2	0.1344
X_1 X_3	0.0604
X_2 X_3	0.0692
R^2	0.9962
Adjusted R^2	0.9739

Significant terms are shown in bold

Fig 29. Response surface plot showing the influence of amount of granulating fluid and amount of binder for fixed level of spheronization speed on dissolution efficiency



3.8 Validation of multiple response optimization model

In order to assess the reliability of the developed mathematical model, formulations corresponding to optimum composition and two additional random compositions covering the entire range of experimental domain were performed. For each of these formulations, the responses were estimated by the use of generated mathematical models and by the experimental procedures. The formulation parameters of the optimum and the random check points, their experimental and predicted values for all the eight response variables are listed in Table 27. The optimised core formulation was prepared with 44.25% w/w of granulating fluid, 2.13% of binder and spheronization carried out at 1000 rpm. The optimised uncoated isoniazid pellets were evaluated for all the physico-mechanical properties using the methods mentioned earlier. The summary of results of various physico-mechanical evaluation parameters and drug content of isoniazid uncoated pellets are shown in Table 28.

Table 27.

The experimental and predicted values for all the eight responses (Y_1 to Y_8) along with percentage prediction error* observed for optimum formulation (A) and random formulation (B and C)

	A (44.24, 2.13, 1000)[#]			B (46.76, 1.7, 1000)[#]			C (42.55, 1.59, 780)[#]		
Response	Experimental value	Predicted value	% PE*	Experimental value	Predicted value	% PE*	Experimental value	Predicted value	% PE*
Y_1	84.95	85.01	-0.07	84.74	85.74	-1.18	87.68	87.21	0.54
Y_2	1021.32	1018.29	0.29	996.11	1000.66	-0.46	955.101	946.835	0.87
Y_3	0.945	0.938	0.74	0.944	0.940	0.42	0.889	0.892	-0.34
Y_4	46.11	45.02	2.36	44.21	45	-1.79	44.00	45.04	-2.36
Y_5	0.485	0.496	-2.26	0.671	0.66	1.64	0.922	0.891	3.36
Y_6	7.32	7.413	-1.27	7.01	6.94	0.99	6.91	7.03	-1.74
Y_7	1.729	1.783	-3.12	1.73	1.77	-2.31	1.86	1.841	1.02
Y_8	68.25	67.35	1.31	67.35	67.01	0.51	67.22	67.78	-0.83

*****Percent prediction error (PE) was calculated using the formula (Experimental Value – Predicted Value) / Experimental Value) x 100.

[#] The values represented in the brackets are the amount of granulating fluid in %w/w, amount of binder in %w/w and the speed of spheronization in rpm, respectively for A, B and C formulations.

Table 28. Summary of results of various micromeritic evaluation parameters and drug content of isoniazid (Coated and Uncoated) pellets

Evaluation Parameter	Isoniazid (Uncoated pellets)	Isoniazid (Coated pellets)
Diameter, d(0.9) (µm)	1436.077 ± 143.72	1604.518 ± 186.80
Roundness Score	1.0257 ± 0.12826	1.0056 ± 0.202
Pellips	0.955 ± 0.0466	0.958 ± 0.0424
Usable yield (%)	94.00	-
Span	0.418	0.528
Friability	< 1%	< 1%
Crushing Strength (N)	6.8 ± 0.72	9.7 ± 1.09
Porosity (%)	42.48 ± 2.75	39.96 ± 1.99
Water Content (%)	1.49 ± 0.75	1.89 ± 0.87
Drug content (%)	95-105	95-105

3.9 Coated delayed release isoniazid pellets

The optimised isoniazid core pellets were prepared using response surface optimization. The optimised batch of core pellets was characterised for its physico-mechanical properties and release characteristics. The physico-mechanical properties of core isoniazid pellets are enlisted in Table 28. The release profile of isoniazid uncoated pellets and coated pellets at different levels of coating (10% , 20% and 30%) is shown in Fig 30. The summary of results of various physico-mechanical evaluation parameters and drug content of isoniazid uncoated pellets are shown in Table 28. To gain greater insight into the morphology of the pellets and surface characterization of coated isoniazid delayed release isoniazid pellets was carried out by SEM (Fig 31).

The drug release from optimised uncoated isoniazid pellets was rapid and high (more than 50% in less than 60 min.) in 0.1 N HCl. This may be due to high water solubility of isoniazid (a borderline BCS Class-I and Class-III) (Fig 30). In order to provide gastric acid resistance and modulate the release of isoniazid from the pellets,

isoniazid pellets were coated with enteric polymers. Gastric acid resistance test is a significant index of drug dissolution performance of an enteric coated formulation. Polymers used for formulating enteric coated formulation should be able to withstand the lower pH values of stomach and be able to disintegrate in the range of pH 6–7. The optimised uncoated isoniazid pellets were coated at three different coating levels (10%, 20% and 30% w/w). Isoniazid release profiles from enteric-coated pellets (with 10%, 20% and 30% polymer weight gain) carried for 2 h in acidic dissolution medium (0.1 N HCl) followed by 1 h in pH 6.8 phosphate buffer as a dissolution media is shown in Fig 29. For pellets coated with 10% and 20% of weight gain isoniazid release ranged from 10% to about 50% in 2 h. Thus, it can be concluded that coating level upto 20% does not provide the gastric acid resistance. Isoniazid pellets coated with higher coating level i.e. 30% w/w, passed the gastric acid resistance test successfully (<10% drug release after 2 h in acidic dissolution medium, USP 2007d). Delayed release isoniazid pellets with 30% enteric coating, when subjected to acid resistance test, released only 9.9% of isoniazid in 2 h in 0.1N HCl (Fig 30) and pellets were found to be completely intact at the end of 2 h. Fig 30. shows that in pH 6.8 isoniazid pellets coated with 30% coating, released 80.60% of isoniazid in 45 min.

Fig 30. Release profile of isoniazid uncoated pellets and coated pellets at different levels of coating (10% , 20% and 30% coating level)

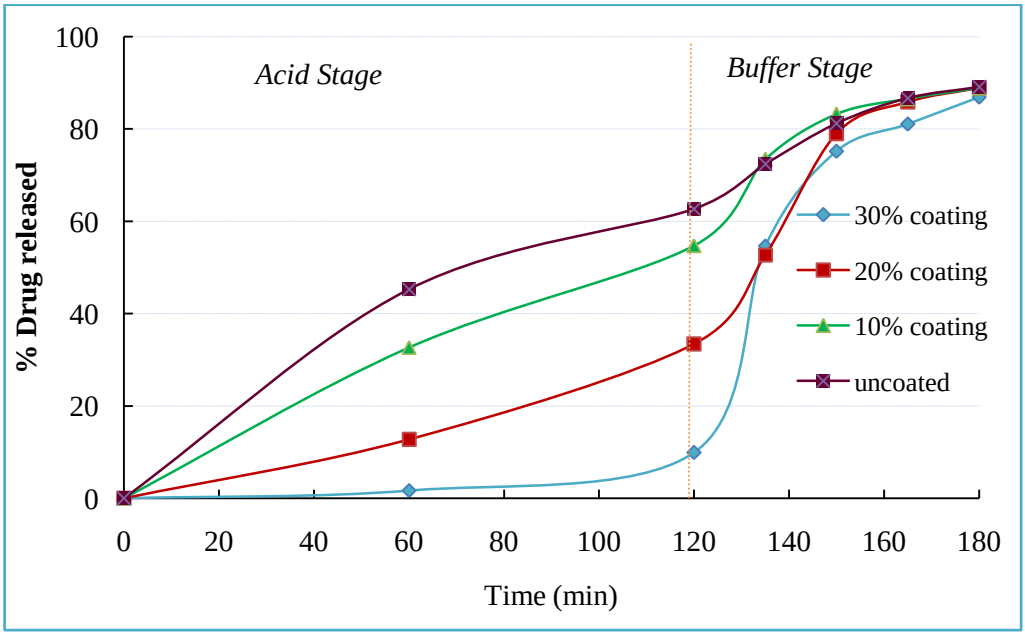
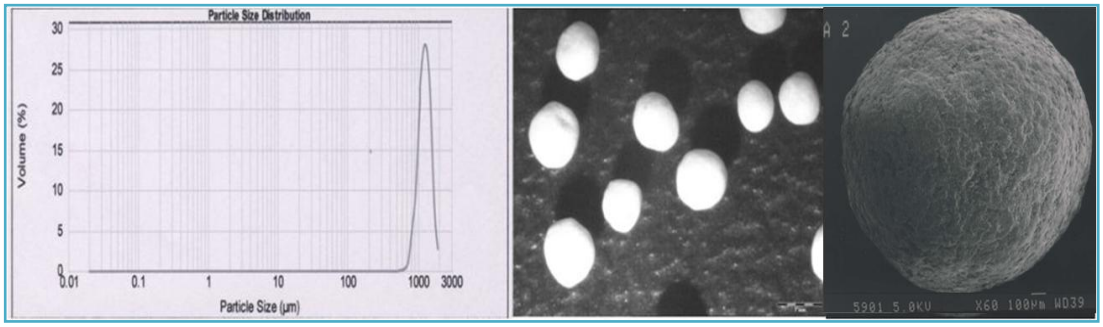


Fig 31. Particle size distribution (by Malvern), Shape characterization (by Image analysis) and Surface characterization (by SEM) respectively, of coated isoniazid pellets



3.10 Stability studies

The optimised isoniazid delayed release pellets were subjected to accelerated stability studies (40°C ± 2°C/75% RH ± 5%) for 6 months. Stability of delayed release isoniazid pellets was also studied at room temperature for 6M. The samples were withdrawn from both the stability conditions at 1 M, 2 M, 3 M and 6 M. The samples were analysed for their water content, drug content and dissolution profile. The water content and drug content of isoniazid delayed release pellets on stability are enlisted in Table 29. The drug content of the isoniazid delayed release pellets on stability ranged from 98-102%. While, the water content of isoniazid delayed release pellets ranged between 1.99-2.65% w/w. An almost constant water uptake and drug content with in pharmacopoeial limits (95%-105% w/w) indicates that isoniazid delayed release pellets are stable till 6M at room temperature and at accelerated stability conditions. The release profile of the stability samples of isoniazid delayed release pellets at room temperature and accelerated stability conditions are shown in Fig 32. It can be concluded from the graph that the gastric acid resistance property of the isoniazid delayed release pellets is not affected on stability. Even after 6M stability the amount release at the end of 120 min. in 0.1N HCl was found to be ~10%, which was similar to that of initial sample of isoniazid delayed release pellets. While ~85% of isoniazid was released in phosphate buffer pH 6.8 within 1 h, even after 6 M stability.

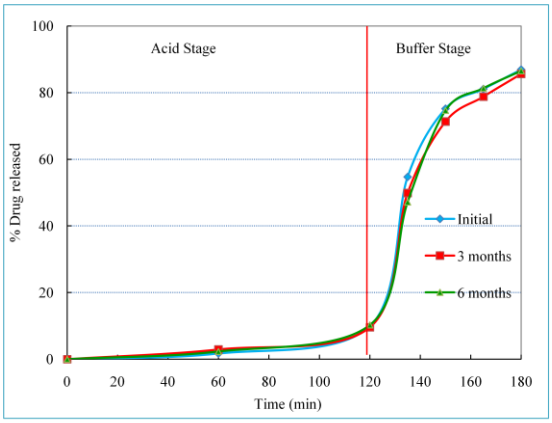
To asses change in release profile statistically, similarity factor (f_2) was calculated. Similarity factor is a fit factors which is essentially a quantitative method, that

reflects the differences between the two release curves (Costa *et.al*., 2003). The f_2 or similarity factor of delayed release isoniazid pellets was found to be > 90 for all the stability samples with respect to their initial release profile. This indicates that release pattern of isoniazid delayed release pellets is not affected by the stability conditions. This might be attributed to reduced water uptake by the coated isoniazid pellets, as indicated by constant moisture content of pellets on stability.

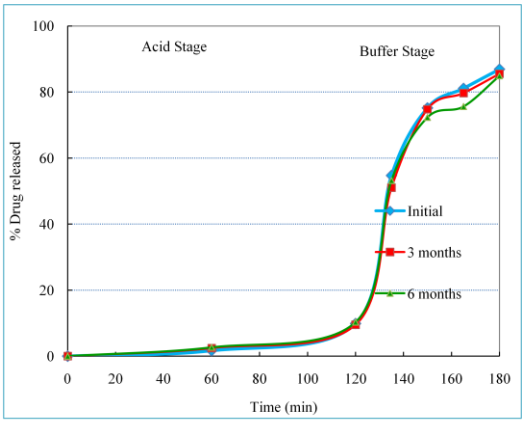
Table 29. Assay and water content of stability samples of delayed release isoniazid pellets at 1 M, 2 M, 3 M and 6 M

		40°C ± 2°C/75% RH ± 5%		At room temperature	
		HDPE	Alu. strip	HDPE	Alu. Strip
Assay (%)	**1M**	99.43	99.99	100.08	100.13
	2M	99.98	99.67	99.98	100.05
	3M	98.55	100.22	99.95	99.89
	6M	100.02	100.05	98.99	100.09
Water Content (%w/w)	**1M**	2.11	2.22	1.97	1.88
	2M	2.47	2.35	2.08	1.99
	3M	2.65	2.55	2.15	2.18
	6M	2.88	2.78	2.29	2.35

Fig 32. Dissolution profile of initial, 3 M and 6 M stability sample of delayed release isoniazid pellets



a. Isoniazid delayed release pellets at 40 °C/75% RH



b. Isoniazid delayed release pellets at room temperature

3.11 Conclusion

Delayed release isoniazid pellets were prepared using extrusion-spheronization followed by coating with functional polymers and were characterized for their performance and the following conclusions were made.

- Delayed release isoniazid pellets were prepared using extrusion-spheronization. The core matrix isoniazid pellets were prepared by extrusion spheronization process. To optimize the core isoniazid pellet, response surface optimization approach was employed.
- The optimized isoniazid core pellets were then coated with enteric polymer, Acryl-EZE® using wurster coating technique. The coating was carried out at three different coating levels (10%, 20% and 30% w/w). Delayed release isoniazid pellets with 30% enteric coating, when subjected to acid resistance test, only 9.9% of isoniazid was released in 2 h in 0.1N HCl and pellets were found to be completely intact at the end of 2 h. This indicates that for imparting gastric acid resistance to a highly water soluble drug like, isoniazid, in pellets 30% w/w of enteric coating is required. While, at pH 6.8, 80.60% of isoniazid was released at the end of 45 min. from the delayed release isoniazid pellets.
- The optimised uncoated and coated isoniazid pellets showed acceptable micromeritic properties (Pellips nearing to 1, Roundness score equivalent to 1, friability less than 1% w/w, yield > 90%). The coated and uncoated isoniazid pellets showed a narrow size distribution as indicated by span values.
- The optimised isoniazid delayed release pellets were subjected to accelerated stability conditions (40°C ± 2°C/75% RH ± 5%) and at room temperature for 6 M. The isoniazid pellets were found to be stable (assay 98-102%; water content 1.88-2.88%).The stability samples of delayed release isoniazid pellets also passed the gastric acid resistance test and released more than 8% drug in phosphate buffer pH 6.8. Isoniazid delayed release pellets showed no significant change in the dissolution profile as indicated by their respective f_2 similarity factor > 90.

References

Almeida-Prieto, S., Blanco-Méndez, J., Otero-Espinar, F.J., 2007. Microscopic image analysis techniques for the morophological charterization of pharmaceutical particles: Influence of the software and the factor algorithms used in the shape factor estimation. Eur. J. Pham. Biopharm. 67, 766-776.

Becker, C., Dressman, J.B., Amidon, G.L., Junginger, H.E., Kopp, S., Midha, K.K., Shah, V.P., Stavchansky, S., Barends, D.M., 2007. Biowaiver monographs for immediate release solid oral dosage forms: Isoniazid, J. Pharm. Sci. 96, 522–531.

Bruce, L.D., Petereit, H., Beckert, T., McGinity, J.W., 2003. Properties of enteric coated sodium valproate pellets. Int. J. Pharm. 264, 85–96.

Chatlapalli, R., Rohera, B.D., 1998. Physical characterization of HPMC and HEC and investigation of their use as pelletization aids. Int. J. Pharm. 161, 179-193.

Chopra, R., Newton, J.M., Alderborn, G., Podczeck, F., 2001. Preparation of pellets of different shape and their characterization. Pharm. Develop. Technol. 6, 495-503.

Costa, P., Lobo, J.M.S., 2001. Modelling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13, 123–133.

Costa, F.O., Sousa, J.J.S., Pais, A.A.C.C., Formosinho, S.J., 2003. Comparison of dissolution profiles of Ibuprofen pellets. J. Control. Rel. 89, 199–212.

Dukic´-Ott, A., Beer, T.D., Remon, J.P., Baeyens, W., Foreman, P., Vervaet, C., 2008. In-vitro and in-vivo evaluation of enteric-coated starch-based pellets prepared *via* extrusion/spheronization. Eur. J. Pharm. Biopharm. 70, 302–312.

Ghebre-Sallassie, I. 1989. Pellets: A General Overview. In: Ghebre-Sallassie, I., (Ed.), Pharmaceutical Pelletization Technology. Marcel Dekker Inc., New York. vol. 37pp. 1-13.

Ghebre-Sellassie, I., Knoch, A., 2007. Pelletization techniques. In: Swarbrick, J.E. (Ed.), Encyclopedia of Pharmaceutical Technology. Informa Healthcare, New York. Vol. 65. pp. 2651-2663.

Gómez-Carracedo, A., Alvarez-Lorenzo, C., Coca, R., Martínez-Pacheco, R., Concheiro, A., Gómez-Amoza, J.L., 2009. Fractal analysis of SEM images and mercury intrusion porosimetry data for the microstructural characterization of microcrystalline cellulose-based pellets. Acta Mater. 57, 295–303.

Gibaldi, M., Pharmacokinetic variability – body weight, age, sex, and genetic factors, In: Lea, F. (Ed.), Biopharmaceutical and clinical pharmacokinetics. Marcel Dekker, Philadelphia, 3rd ed., pp. 220-224.

Howard, M.A., Neau, S.H., Marvin, J.S., 2006. PEO and MPEG in high drug load extruded and spheronized beads that are devoid of MCC. Int. J. Pharm. 307, 66-76.

Holdiness, M.R., 1984. Clinical pharmacokinetics of antitubercular drugs. Clin. Pharmacokinet. 9, 511-544.

Hurwitz, A., Scholzman, D.L., 1974. Effects of antacids on gastrointestinal absorption of isoniazid in rat and man. Am. Rev. Resp. Dis.1386-1388.

ICH harmonised tripartite guideline, 2003. Stability testing of new drug substances and products Q1a (R2), 4th version.

Jayaram, R., Shandil, R.K., Gaonkar, S., Kaur, P., Suresh, B.L., Mahesh, B.N., Jayashree, R., Nandi, V., Bharath, S., Kantharaj, E., Balasubramanian, V., 2004. Isoniazid pharmacokinetics pharmacodynamics in an aerosol infection model of tuberculosis. Antimicrob. Agents Chemother. 48, 2951-2957.

Joshi, A., Pund, S., Nivsarkar, M., Vasu, K., Shishoo, C.J., 2008. Dissolution test for site-specific release isoniazid pellets in USP Apparatus 3 (reciprocating cylinder): Optimization using Response Surface Methodology. Eur. J. Pharm. Biopharm. 69, (2), 769-775.

Kagan, L., Hoffman, A., 2008. Systems for region selective drug delivery in the gastrointestinal tract: biopharmaceutical considerations. Expert Opin. Drug Deliv. 5(6), 681-692.

Koo, O.M.Y.K., Heng, P.W.S., 2001. The influence of microcrystalline cellulose grade on shape and shape distributions of pellets produced by extrusion- spheronization. Chem. Pharm. Bull. 49, 1383-1387.

Kramer, J., Blume, H., 1994. Biopharmaceutical aspects of multiparticulates. In: Sellassie I.G. (Ed.), Multiparticulate Oral Drug Delivery. Marcel Dekker, Inc., New York. pp. 307–355.

Lustig-Gustafsson, C., Johal, H.K., Podczeck, F., Newton, J.M., 1999. The influence of water content and drug solubility on the formulation of pellets by extrusion and spheronization. Eur. J. Pharm. Sci. 8, 147–152.

Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of Rifampicin and Isoniazid (alone and their combination) in the rat. Int. J. Tuber. Lung Dis. 7, 797-803.

Mehta, A.M., 1989. Evaluation and characterization of pellets. In: Ghebre-Sallassie, I. (Ed.), Pharmaceutical Pelletization Technology, vol. 37. Marcel Dekker Inc., New York, pp. 241–265.

Melia, C.D., Washington, N., Wilson, C.G., 1994. Advantages and disadvantages of multiparticulate delivery systems, In: Melia, C.D., Washington, N., and Wilson C.G. (Eds.), Multiparticulate oral dosage forms: Technology and Biopharmaceutics. Scottish Academic Press, Edinburgh, pp. 135-140.

Menegola, J., Steppe, M., Schapoval, E.E.S., 2007. Dissolution test for citalopram in tablets and comparison of *in vitro* dissolution profiles. Eur. J. Pharm. Biopharm. 67, 524–530.

Newton, J.M., Pinto, M.R., Podczeck, F., 2007. The preparation of pellets containing a surfactant or a mixture of mono- and di-glycerides by extrusion/spheronization. Eur. J. Pharm. Sci. 30, 333-342.

Petri, W.A., 2001. Antimicrobial Agents. In: Hardmann, J.G., Limbird, L.E., Gilman, A.G. (Eds.), The Goodman and Gilman's: the pharmacological basis of therapeutics. MacGraw Hill Medical Publishing division, New York, 10th ed., pp. 1273-1294.

Podczeck, F., Rahman, S.R., Newton, J.M., 1999. Evaluation of a standardised procedure to assess the shape of pellets using image analysis. Int. J. Pharm. 192, 123–138.

Rastogi, R., Sultana, Y., Aqil, M., Ali, A., Kumar, S., Chuttani, K., Mishra, A.K., 2007. Alginate microspheres of Isoniazid for oral sustained drug delivery, Int. J. Pharm. 334, 71–77.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., 2001. Impaired Bioavailability of Rifampicin from its Fixed Dose Combination (FDC) Formulations with Isoniazid, Indian J. Pharm. Sci., 63(6), 443-449.

Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.

Sousa, A., Podczeck, F., Newton, J.M., 2002. Factors influencing the physical characteristics of pellets obtained by extrusion-spheronization. Int. J. Pharm. 232, 91-106.

Steckel, H., Mindermann-Nogly, F., 2004. Production of chitosan pellets by extrusion/ spheronization. Eur. J. Pharm. Biopharm. 57, 105-114.

US Pharmacopoeia 30/NF25, 2007a. US Pharmacopoeial Convention, Rockville, MD, pp. 242.

US Pharmacopoeia 30/NF25, 2007b. US Pharmacopoeial Convention, Rockville, MD, pp. 385.

US Pharmacopoeia 30/NF25, 2007c. US Pharmacopoeial Convention, Rockville, MD, pp. 2413.

US Pharmacopoeia 30/NF25, 2007d. US Pharmacopoeial Convention, Rockville, MD, pp. 282.

WHO. 2003. Treatment of tuberculosis: Guidelines for national programmes, 3rd Edition.

Zhang, Y., (2003). Isoniazid. In: Rom, W.N., Garay, S.M. (Eds.), Tuberculosis. 2nd ed., Lippincott Williams & Wilkins, Philadelphia, PA, pp. 739 58.

Chapter 4

Stability & Human bioavailability of novel rifampicin and isoniazid FDC

"Every great advance in science has issued from a new audacity of imagination"
.....John Dewey

4

Stability and human bioavailability study of novel rifampicin and isoniazid FDC

4.1 Introduction

TB is a major health problem in the developing countries like India, which has the maximum pool of TB patients. As of now the only available treatment lies in the effective utilization of few available anti-TB drugs, especially rifampicin and isoniazid. However, the emergence of resistant strains has come as a major 'bottleneck' in containing the spread of TB and its treatment. Combination of drugs can effectively counter this problem that led to the concept of FDCs. At the same time, it is very important to ensure that the bioavailability of the drugs combined in the FDCs is not compromised. This is particularly true for rifampicin where there are ample reports of reduced bioavailability from FDCs (Agrawal *et.al*., 2002). Rifampicin is a critical component in the therapeutic armamentarium for tuberculosis, and more recently for treating opportunistic infections associated with the acquired immune-deficiency syndrome (AIDS). The problem of reduced bioavailability of rifampicin from FDC products of anti-tuberculosis drugs is a matter of global concern. An integral part of the strategy to fight the disease is 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 9 mg/kg body weight can result in therapeutic failure (Long *et.al*., 1979) and hence can be the cause of development of drug resistance. The problems associated with quality of FDC products are in the current focus.

Over the years, two serious problems have been reported with rifampicin and isoniazid FDCs that includes (Laserson *et.al*., 2001; Shishoo *et.al*., 2001; Immanuel *et.al*., 2003; Singh and Mohan, 2003; Bhutani *et.al*., 2004; Luyen *et.al*., 2005):

- The impaired and variable bioavailability of rifampicin from FDC formulations with isoniazid
- Poor stability of rifampicin containing FDCs

The use of substandard FDC ultimately results in drug resistant TB and treatment failure (Panchagnula *et.al*., 1999; IUTALD/ WHO, 1994). 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*., 2000; Shishoo *et.al*., 2001). In this backdrop, both WHO and IUATLD recommend the use of FDCs proven bioavailability of rifampicin (IUTALD/WHO, 1999; Panchagnula *et.al*., 2003).

It is thus expected that bioavailability concerns associated with rifampicin could be overcome by developing a system that attains segregated delivery of the two drugs, with rifampicin released immediately in the stomach and isoniazid in the small intestine (through development of an enteric-release system), thus targeting 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 that, in the present study a novel formulation was designed and developed 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 present chapter will cover the stability studies of the developed novel anti-TB FDC at room temperature and accelerated conditions. The oral bioavailability of novel rifampicin and isoniazid FDC, using a commercially available rifampicin and isoniazid FDC tablet as reference will also be covered in this chapter.

4.2 Stability studies of rifampicin and isoniazid FDC

4.2.1 Methods

The weighed amount of rifampicin pellets, a rifampicin tablet and weighed amount of isoniazid was filled an empty hard gelatine capsule. The capsules prepared were subjected for stability studies at room temperature and at accelerated stability condition. The capsules were stored in a tightly closed HDPE container and aluminium (Alu.) packs. The stability studies were carried out at room temperature and accelerated relative humidity conditions as per ICH guidelines. The accelerated relative humidity conditions were 40°C ± 2°C/75% RH ± 5% RH (ICH, 2003). The stability samples were withdrawn and analysed at 1, 2, 3 and 6 M for drug content, water content and release profile. The assay, water content and dissolution studies of the floating rifampicin tablet

were carried as per the methods described earlier. To ensure the equivalence in release profile of the stability samples with that of initial samples, the fit factor, f_2, was calculated. The formula used for calculating f_2 values is shown in Eq. (27):

$$f_2 = 50 \log\{[1+ 1/n\sum_{t=1}(R_t-T_t)]^{0.5} \text{ X } 100\} \qquad \text{--(27)}$$

4.3 Bioavailability studies of rifampicin and isoniazid FDC in human volunteers

4.3.1 Materials

R-Cinex tablets (Lupin Pharmaceuicals Limited, Pune, India) were procured from the market and were used as a reference product. Novel rifampicin isoniazid FDC capsules were prepared as previously described and packed in aluminum bags, sealed and labelled with full composition, batch number. Papaverine hydrochloride and pyrizinamide was kindly gifted by Biologicals E. Ltd, India and Macleods Pharmaceuticals, India. Dichloromethane, methanol, potassium dihydrogen orthophosphate and acetonitrile were obtained from Qualigens (Delhi, India). All reagents were of analytical or high performance liquid chromatography (HPLC) grade and are enlisted in Annexure 1.

4.3.2 Methods

4.3.2.1 Clinical protocol

An open label, balanced, randomised, three-treatment, three-sequence, three period, crossover, single centre bioavailability study of single oral dose of fixed dose combination of rifampicin and isoniazid in twelve healthy, adult, male, human subjects under fasting conditions was carried out. The study was performed at the B. V. Patel PERD Centre, Ahmedabad. The study protocol was approved by the Insitutional Ethics Committee of B. V. Patel PERD Centre. The study was conducted in accordance with the Declaration of Helsinki ethical principles (WMA, October 2008).

Subjects underwent a screening 14 days prior to the day of first dosing. Volunteers gave a written informed consent after receiving a detailed explanation of the investigational nature of the study. They were non-smokers, and were judged healthy on the basis of medical history, physical examination, electrocardiogram and investigation of biochemical, immunological, parasitological and haematological parameters in blood and urine.

Upon entering into the study, the subjects were housed in the clinical facility of the trial site for 10-12 h, prior to dosing till 24 h post dose in each of the three periods. All the subjects were fasted overnight, at least 12 h, before scheduled time for the dose administration. A standardised meal was given at 4 h and 12 h post dose in each period. During housing, meal plans for all the periods was same.

Prior and concomitant therapy

All the subjects were abstained from intake of medication from two weeks prior and during the study. They were asked to abstain from beverages containing alcohol or quinine between 24 h before and 48 h after drug dosing per experimental period. Drinking of water was allowed up to 2 h before drug administration. The subjects fasted for at least 10 h before administration of the medication.

Procedure

Before drug administration, an intravenous canula was placed in an antecubital vein and kept patent by use of a saline solution. The drug was administered with a glass of water (about 240 ml). From 2 h after dosing, intake of water was allowed. A standard lunch and dinner were served at 4 and 10 h post-dosing, respectively.

Blood sampling

Venous blood samples (6 ml) were taken before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12 and 24 h after drug intake. Exact times of blood sampling were noted in the case report Form. Blood samples were collected in prelabelled heparinised tubes and centrifuged for 7 min., at 4000±100 rpm, at a temperature below 4ºC within 2 h after collection for collecting the plasma. Separated plasma was aspirated with a disposable pipette and transferred to plastic plasma vials containing ascorbic acid. The plasma vials were sealed and labelled with the mentioning project number, subject number, period, sampling time point, and sample number and stored at -20°C for interim storage and at -80°C until assay of rifampicin and isoniazid by HPLC.

Randomisation

The study was conducted in a randomized crossover design. Subjects were randomly assigned to receive a single dose of 450mg rifampicin and 300 mg isoniazid. A washout period of 1 week separated both drug intakes. Subjects entering the study were allocated a number from 1 to 12 and were administered medication as per the randomization schedule.

4.3.3 Determination of rifampicin in plasma

Rifampicin concentrations were determined by a validated HPLC method: 0.5 ml plasma was spiked with 50 µl aqueous ascorbic acid solution (10 µg/ml) and 9 µg internal standard, papaverine HCl in methanol. Samples were then buffered with 0.5 ml of 0.005M K_2HPO_4 containing 1 µg/ml ascorbic acid (pH 7), and extracted with 6 ml of dichloromethane. The organic layer was transferred to conical centrifuge tubes and evaporated until dry, under a gentle nitrogen stream. The residue was redissolved in 100 µl mobile phase containing ascorbic acid (50 mg/l), and a 50 µl aliquot was injected onto a 5 µm particle size, reverse phase, C-18, Qualisil column (250 X 3.9 mm).

Chromatographic conditions

The HPLC equipment consisted of a solvent pump (Jasco PU 980, Tokyo, Japan) set at a constant flow rate of 1.00 ml/min, a UV detector (Jasco UV 875, Jasco, Tokyo, Japan) set at 320nm wavelength, a C-18 reversed phase precolumn and Base deactivated (BDS) column Kromasil (LCGC, USA) and an automatic integration system (Borwin, Japan). The mobile phase was based on the composition described by Pargal and Rani (2001). It consisted of a filtered and degassed mixture of 45% acetonitrile and 55% of 0.01M KH_2PO_4 (pH 6.5). The method was validated as per ICH guidelines and was found to be specific, accurate, linear in the concentration range of 20 to 0.5 µg/ml, limit of quantitation was 0.5 µg/ml and limit of detection was 0.1 µg/ml (Pund, 2010).

4.3.4 Determination of isoniazid in plasma

Isoniazid concentrations were determined by a validated HPLC method: 200µl of pyrazinamide (Internal standard) solution in acetonitrile was added to 20 µl plasma and centrifuged for 10 min at 10,000 rpm. The supernatant was collected and dichloromethane was added to it. This mixture was then centrifuged for 10 min at 10,000 rpm and 100 µl of aqueous phase was collected. 50 µl of the sample was then diluted with an equal amount of mobile phase and injected onto 5 µm particle size, reverse phase, C-18 BDS column (250 X 3.9 mm).

Chromatographic conditions

The HPLC equipment consisted of a solvent pump (Jasco PU 980, Jasco, Tokyo, Japan) set at a constant flow rate of 1.00 ml/min, a variable wavelength detector (Jasco UV 875, Jasco, Tokyo, Japan) set at 264 nm wavelength, a C-18 reversed

phase precolumn and Base deactivated (BDS) column Kromasil (LCGC, USA) and an automatic integration system (Borwin, Japan). Mobile phase consisted of a filtered and degassed mixture of 3.5% acetonitrile and 97.5% of 0.01M KH_2PO_4 buffer. The method was validated as per ICH guidelines and was found to be specific, accurate, linear in the concentration range of 20 to 0.5 µg/ml, limit of quantitation was 0.5 µg/ml and limit of detection was 0.1 µg/ml (Pund, 2010).

4.3.5 Statistical analysis

4.3.5.1 Pharmacokinetic Analysis

Maximal plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) were obtained directly by the visual inspection of each subject's plasma concentration-time profile. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (K_{el}). The elimination half-life was obtained from the formula,

$$t_{1/2} = \ln(2)/ K_{el} \quad \text{--(28)}$$

Where 'ln' is the natural logarithm.

The Area Under Curve $(AUC)_{0-t}$ from time zero to the last quantifiable point (C_t) was calculated using the trapezoidal rule. The area under the plasma concentration-time from 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant.

For any AUC computation, concentration at time point't' (C_t) values below limit of quantification (LOQ) was set to zero. (C_t) values below (LOQ) were to be ignored in the linear regression analysis. Statistical analysis for evaluating bioequivalence were carried out on logarithmically (natural) transformed pharmacokinetic parameters of rifampicin and isoniazid (C_{max} and AUC_{0-t}). The parameter T_{max} was analyzed on untransformed data.

4.3.5.2 Descriptive statistics

Descriptive statistics of C_{max} and AUC_{0-t} for test (novel rifampicin isoniazid FDC) and reference (marketed rifampicin isoniazid FDC) products were calculated. The individual

values of these endpoints together with descriptive statistics and ratios B/A were tabulated for test and reference formulations of rifampicin and isoniazid.

4.3.5.3 Analysis of variance

The ln-transformed pharmacokinetic parameters (C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$) were analyzed using an ANOVA model with the main effects of sequence, subject nested within sequence, period and formulation.

4.3.5.4 90% Confidence interval

For the pharmacokinetic parameters C_{max} and AUC_{0-t}, 90% confidence intervals for the ratios of test and reference product averages were calculated using the ANOVA of the In-transformed data. Consistent with the two one sided test for bioequivalence, 90% confidence interval for the ratio of both the products averages were calculated by first calculating the 90% confidence interval for the differences in the averages of the ln-transformed data and then taking the antilogarithms of the obtained confidence limits.

4.3.5.5 Bioequivalence criteria

Bioequivalence was evaluated using average bioequivalence approach; this is based on the ratio of average In-transformed responses. The 90% confidence interval for ratio of average In-transformed C_{max}, AUC_{0-t} of rifampicin and isoniazid was the basis for concluding the equivalence of product A and B. The 90% CI should hence lie within the bioequivalence limit (80.00-125.00).

4.4 Results and discussion

4.4.1 Stability studies of rifampicin and isoniazid FDC

The rifampicin and isoniazid FDC capsules were subjected to stability studies at room temperature and at the accelerated stability conditions (40°C ± 2°C/75% RH ± 5%) for the duration of 6 M. The samples were withdrawn at 1 M, 2 M, 3 M and 6 M and analysed for their water content, drug content and the dissolution profile. The water content and drug content in rifampicin isoniazid FDC on stability are shown in Table 30. The drug content of the rifampicin and isoniazid from the FDC on stability was found to be in the range between 98.99-100.65% and 98.55-100.05%, respectively. While, the water content of rifampicin –isoniazid FDC ranged between 4.05- 4.52% w/w. According to the ICH guidelines on stability testing, if ‘significant change’ occurs at any

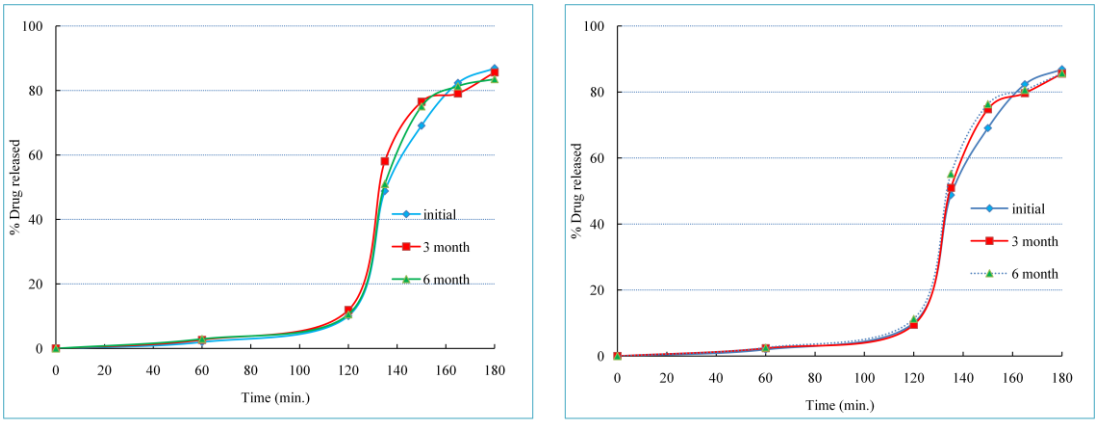
time during 6 M of testing at the accelerated storage conditions of 40°C ± 2°C/75% RH ± 5%. The 'significant change' for a solid drug product is defined as: a 5% change in assay from its initial value; or failure to meet the acceptance criteria for appearance, physical attributes and dissolution test (ICH, 2003). Hence, it can be concluded that no significant change was observed in FDC after 6 M with respect to its water content and drug content.

The release profile of isoniazid from the stability samples of novel FDC at room temperature and accelerated stability conditions are shown in Fig 33. It can be concluded from the graph that the gastric acid resistance property of the isoniazid delayed release pellets had no affect on the stability. Even after 6 M stability the amount release at the end of 120 min. in 0.1N HCl was found to be ~10%, which was similar to that of initial sample of isoniazid delayed release pellets. While ~85% of isoniazid was released in phosphate buffer pH 6.8 within 1 h, even after 6 M stability.

Table 30. Assay and water content of stability samples of rifampicin- isoniazid pellets

40°C ± 2°C/75% RH ± 5%				At room temperature	
		HDPE	**Alu. Strip**	**HDPE**	**Alu. Strip**
Assay of Rifampicin (%)	**1M**	99.35	100.04	99.76	100.65
	2M	100.08	98.99	99.65	100.99
	3M	100.45	100.23	100.32	99.18
	6M	99.87	99.99	100.56	100.14
Assay of Isoniazid (%)	**1M**	99.43	99.99	100.08	100.13
	2M	99.98	99.67	99.98	100.05
	3M	98.55	100.22	99.95	99.89
	6M	100.02	100.05	98.99	100.09
Water Content (%w/w)	**1M**	4.21	4.05	4.05	4.18
	2M	4.34	4.46	4.52	4.32
	3M	4.39	4.38	4.47	4.27
	6M	4.18	4.32	4.33	4.08

Fig 33. Dissolution profile of initial, 3 M and 6 M stability sample of isoniazid from rifampicin-isoniazid FDC

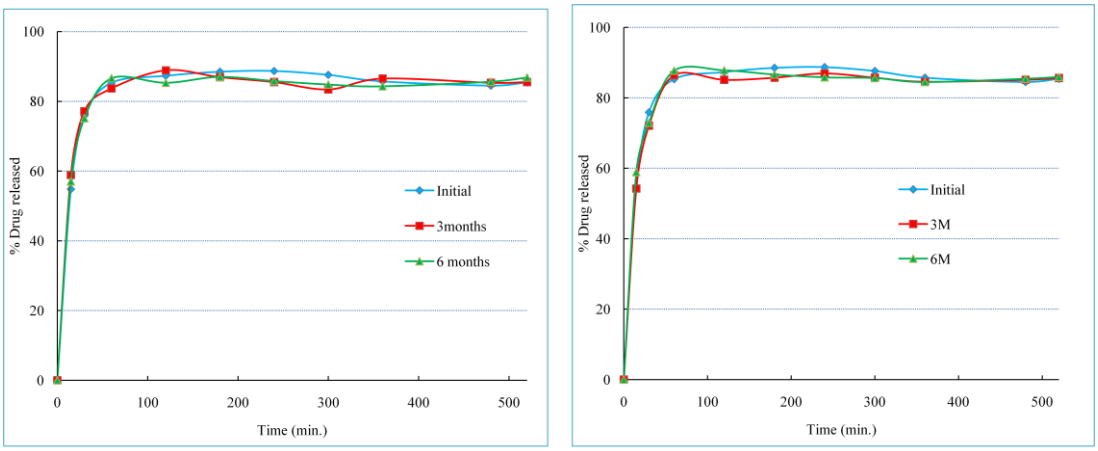


a. Rifampicin-Isoniazid FDC at 40 °C/ 75% RH

b. Rifampicin-Isoniazid FDC at room temperature

The release profile of rifampicin from the stability samples of rifampicin and isoniazid FDC capsules at room temperature and accelerated stability conditions are shown in Fig 34. It can be seen in the graphs that the amount of rifampicin released from the initial samples was 85±2.79% at the end of 520 min., while the FDC samples after 6 M showed a release of 85.2 ± 1.78% of rifampicin.

Fig 34. Dissolution profile of initial, 3 M and 6 M stability sample of rifampicin from rifampicin-isoniazid FDC



a. Rifampicin-Isoniazid FDC at 40 °C/ 75% RH

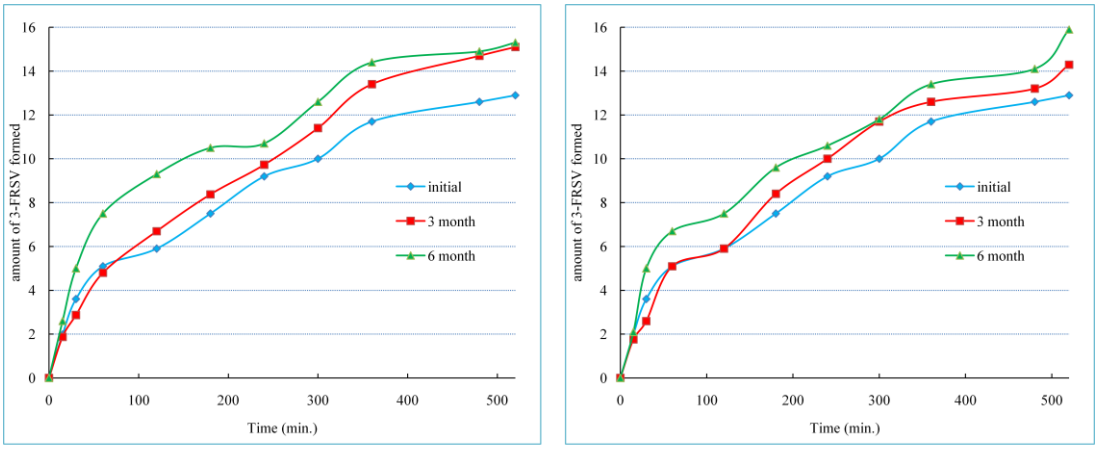
b. Rifampicin-Isoniazid FDC at room temperature

However, rifampicin follows a pH dependent degradation pattern. In the acidic medium, rifampicin hydrolyzes to 3-Formyl rifamycin SV (3-FRSV) which gets precipitated in acidic conditions. The 3-FRSV formed shows high *in vitro* antimicrobial activity but is inactive *in vivo* (Savale, 2003). Therefore, formation of 3-FRSV in the acidic environment of stomach is an important factor affecting bioavailability of rifampicin and cannot be overlooked (Shishoo *et.al.*, 1999).

Shishoo *et.al*., 1999, evaluated various marketed FDCs of rifampicin isoniazid and found that there is a significant increase in formation of 3-FRSV in presence of isoniazid. This indicates that the presence of isoniazid catalyzes degradation of rifampicin to 3-FRSV in 0.1 N HCl. Also, with the increase in time the formation of 3-FRSV increases. Hence, it was essential to estimate the amount of 3-FRSV formed from the novel FDC of rifampicin and isoniazid. The amount of 3-FRSV formed from novel FDC was compared with the market sample of rifampicin and isoniazid FDC.

The amount of 3-FRSV formed in rifampicin-isoniazid FDC on stability is shown in Fig 35. A comparison of amount of 3-FRSV formed from market FDC and novel FDC of rifampicin and isoniazid is shown in Table 34.

Fig 35. Formation of 3-FRSV in dissolution samples of initial, 3 M and 6 M stability sample of rifampicin-isoniazid FDC



a. Rifampicin-Isoniazid FDC at 40 °C/ 75% RH

b. Rifampicin-Isoniazid FDC at room temperature

The amount of 3-FRSV formed at the end of 520 min. was found to be around 12.9% as against 25.76% of 3-FRSV formed from the market FDC of rifampicin and isoniazid. The amount of 3-FRSV formed from novel anti-TB FDC was almost 40.61% less than that of the amount formed from the marketed sample. While after 6 M, the amount of 3-FRSV formed increased from 12.9% to 15.3% only.

This is in conjunction with the earlier study done by Shishoo *et.al*, 1999, carried out to evaluate the dissolution pattern of the various market FDC of rifampicin isoniazid and found that a maximum of 21% of 3-FRSV was formed in 45 min. from the market sample of rifampicin –isoniazid FDC during dissolution studies in 0.1N HCl.

Table 31. Amount of 3-FRSV formed in 0.1N HCl from the market FDC and novel FDC on stability

Time (min.)	Market FDC	Novel FDC (initial)	Novel FDC (3M)	Novel FDC (6M)
15	5.98	1.99	1.87	2.6
30	7.58	3.6	2.87	5
60	12.36	5.1	4.8	7.5
120	14.54	5.9	6.7	9.3
180	15.43	7.5	8.38	10.5
240	17.09	9.2	9.73	10.7
300	19.93	10	11.4	12.6
360	21.36	11.7	13.4	14.4
480	23.32	12.6	14.7	14.9
520	25.76	12.9	15.1	15.3

In contrast, the amount of 3-FRSV formed during dissolution studies of the developed novel rifampicin-isoniazid FDC in 60 min. was found to be around 5.1% from initial sample and 7.5% after 6 M stability. The developed novel rifampicin isoniazid FDC after 6 M showed only an increase of 2.4% of 3-FRSV at the end of 520 min. (Table 31), indicating that the developed FDC formulation is stable.

Earlier studies have confirmed the enhanced degradation of rifampicin in presence of isoniazid (Shishoo *et.al*, 1999; Singh *et.al*., 2000). An elegant mechanism to explain increased degradation of rifampicin in acidic conditions in presence of isoniazid has been suggested. The reaction mechanism can be represented as below-

Rifampicin + H_2O $\rightleftharpoons$ 3-FRSV + 1-amino 4-methyl piperazine

Isoniazid + 3-FRSV $\rightleftharpoons$ Isonicotinyl hydrazone + H_2O

It has been found that subsequent to hydrolysis of rifampicin to 3-FRSV, isoniazid reacts with 3-FRSV to form isonicotinyl hydrazone of 3-FRSV in a reversible manner where the forward reaction is faster second order reaction while the backward reaction is a slower first order reaction. The overall reaction is favoured towards formation of hydrazone and hence, an overall increase in degradation of rifampicin to 3-FRSV is observed. At the same time, hydrazone are known to hydrolyse in the acidic medium resulting in regeneration of isoniazid and 3-FRSV. This indicates that isoniazid remains

unaffected and plays a role of a "catalyst" in the degradation of rifampicin in acidic conditions to 3-FRSV (Savale, 2003; Singh, *et.al*., 2006). And hence a higher degradation or formation of 3-FRSV was found in the case of market samples, wherein both rifampicin and isoniazid are released together in the acidic medium and facilitate the formation of hydrazone *via* formation of 3-FRSV.

While in case of novel FDC, 41% reduction in the formation of 3-FRSV can be attributed to the segregated release of rifampicin and isoniazid. In acidic medium, only rifampicin is released and not the isoniazid. The isoniazid is not released in acidic medium since it has been coated with enteric polymers, which provide the protection to isoniazid from being released in acidic medium. As a result of the absence of isoniazid in the acidic medium, the degradation of rifampicin is not favoured in the forward direction and hence there is a reduced formation of 3-FRSV in acidic medium. Here it is worthwhile to note that, the amount and profile of formation of 3-FRSV from the novel FDC of rifampicin isoniazid is not affected at accelerated stability conditions, even after 6 M (as shown in Fig 35 and Table 31).

It can be hence concluded that even after 6 M, rifampicin and isoniazid are found to be stable in the developed novel FDC. Also, minimal decomposition of rifampicin *in vitro*, thus provides a proof of the concept that formulating FDC rifampicin and isoniazid with segregated site of drug delivery results in improved stability of rifampicin in the FDC.

Additionally, to asses change in release profile statistically, similarity factor (f_2) was also calculated. To statistically confirm the similarity between the release profiles, f_2, factor was calculated. The f_2 factor for rifampicin release profile was found to be >85, while, for isoniazid f_2> 90. The f_2 value above, 50 is indicative of similarity between the two release profiles compared.

Thus, it can be concluded that the release of rifampicin and isoniazid from the novel developed rifampicin isoniazid FDC is similar and is not significantly affected by the higher humidity and temperature conditions and are stable at accelerated stability conditions.

Based on non-significant change in uptake of water and drug content and f_2 values indicates that novel FDC of rifampicin and isoniazid is stable till 6 M at room

temperature and at accelerated stability conditions. Such formulations would rule out the possibility of failure in the performance of formulations due to stability-related problems during distribution and handling and especially in region IV countries. Region IV countries experience high temperature and humidity and are mainly categorised as TB high-burden countries like India, South Africa etc.

4.4.2 Bioavailability studies

Using the proposed sensitive and specific HPLC method, plasma levels of rifampicin and isoniazid were monitored after administration of rifampicin and isoniazid FDC formulation to human volunteers. Typical chromatograms showing peaks for rifampicin and isoniazid in human plasma samples of a volunteer collected at various time points after administration of rifampicin and isoniazid FDC are shown in Fig 36 and 37, respectively.

Fig 36. A typical chromatogram of rifampicin analysed in human plasma along with papaverine hydrochloride (internal standard)

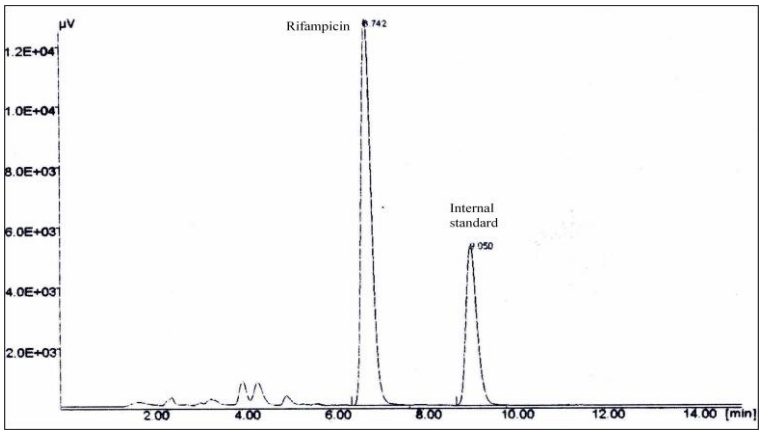
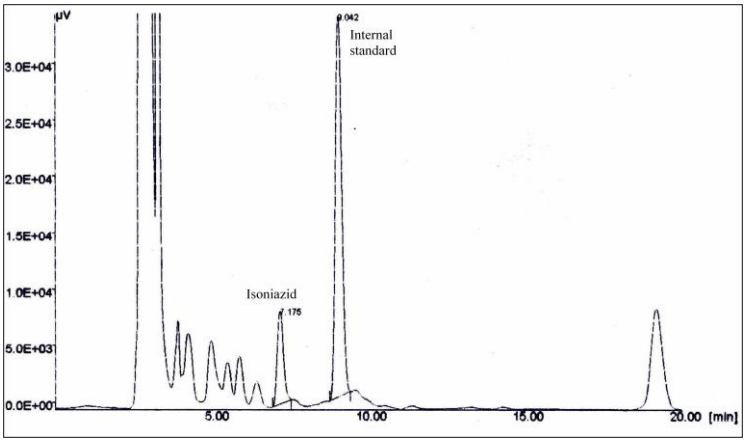
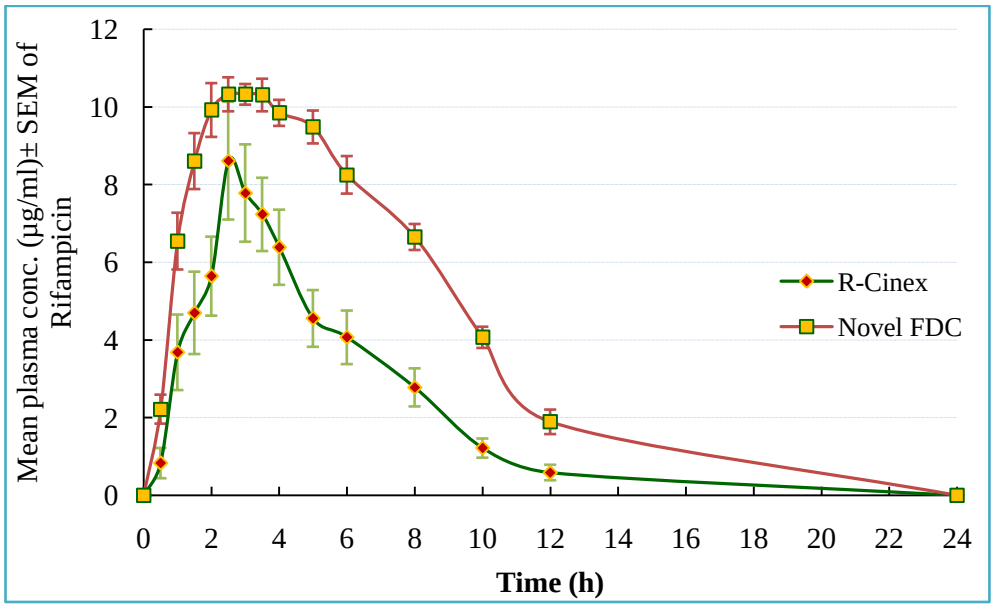


Fig 37. A typical chromatogram of isoniazid analysed in human plasma along with pyrazinamide (internal standard)



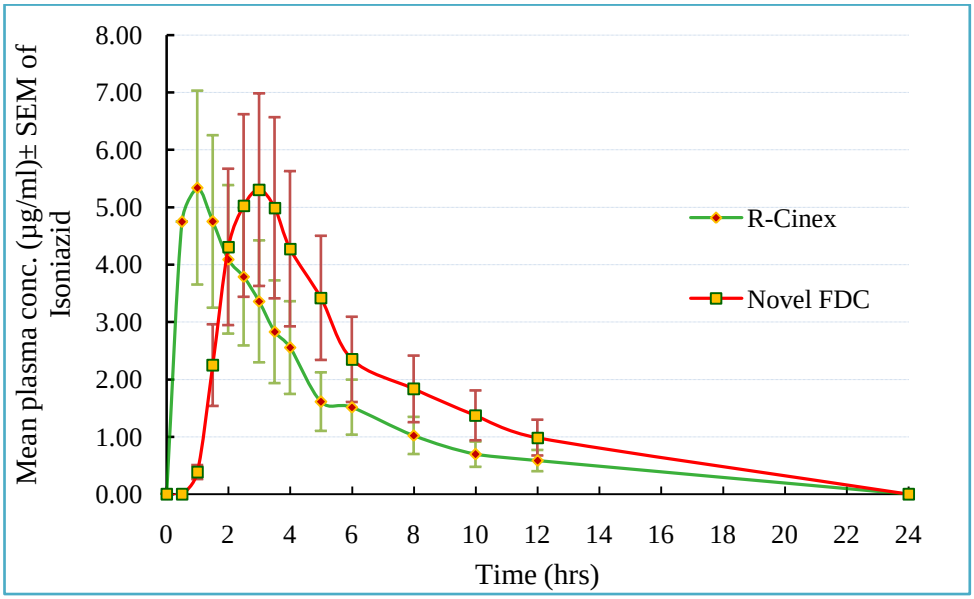
Comparative plasma concentration-time profiles of rifampicin, after administration of rifampicin and isoniazid FDC, novel FDC and marketed FDC formulation are shown in Fig 38. Comparative plasma profiles of rifampicin after administration of rifampicin and isoniazid FDC, novel FDC reveals, an increase in plasma levels of rifampicin from the novel FDC formulation in comparison to marketed FDC formulation (Fig 38).

Fig 37. Mean plasma rifampicin concentration (µg/ml) versus time profile of isoniazid for novel FDC and market FDC of rifampicin and isoniazid in healthy male subjects under fasting conditions



Similarly, comparative plasma concentration-time profiles of isoniazid, after administration of rifampicin and isoniazid FDC, novel FDC and marketed FDC formulation are shown in Fig 39. Plasma profile of isoniazid from novel rifampicin isoniazid FDC formulation clearly shows a lag time of 2 h followed by a rapid absorption of isoniazid. Whereas, in case of marketed FDC formulation an immediate absorption of isoniazid can be seen (Fig 39).

Fig 39. Mean plasma isoniazid concentration (µg/ml) versus time profile of isoniazid for novel FDC and market FDC of rifampicin and isoniazid in healthy male subjects under fasting conditions



Various pharmacokinetic parameters, mainly C_{max}, K_{el}, T_{max}, $T_{1/2}$ and AUC $_{0-\infty}$ for rifampicin and isoniazid obtained after administration of novel rifampicin isoniazid FDC formulation of rifampicin isoniazid FDC were determined by subjecting the respective plasma concentration-time data to noncompartmental analysis and are summarized in Table 32 and 33.

Bioequivalency is the most important quality control tool as a surrogate for the therapeutic efficacy. The rate and extent measures become surrogate indicators of therapeutic outcome to assess the drug product performance. The maximum plasma concentration (C_{max}) and the time of its occurrence (T_{max}) are thought to be reasonable measures for rate of absorption. The determination of the area under the concentration–time curves (AUCs) is the method most commonly used by regulatory agencies to assess the extent of drug absorption after single-dose administration of oral products (Panchagnula *et.al*., 2006).

The average C_{max} value (representing the rate of absorption) for rifampicin after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed FDC formulation, was found to be 10.27 µg/ml and 8.64 µg/ml, respectively (as shown in Table 32). Thus, it can be concluded that an increase of about 18.87% was observed in C_{max} after administration of novel rifampicin-isoniazid FDC in comparison to the marketed FDC.

Table 32. Summary of pharmacokinetic parameters of rifampicin plasma profile, after administration of the market FDC vs novel FDC formulations

	Formulation A (Market FDC)						Formulation B (Novel FDC)					
	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)	$T_{1/2}$ (h)	K_{el} (h^{-1})	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)	$T_{1/2}$ (h)	K_{el} (h^{-1})
Mean	8.64	2.30	46.64	46.63	0.77	1.05	10.27	3.00	92.58	92.58	3.00	1.09
SD	3.85	0.86	24.01	23.99	0.39	0.35	0.72	1.00	13.70	13.70	1.00	0.21
SEM	1.22	0.27	7.59	7.59	0.12	0.11	0.23	0.32	4.33	4.33	0.32	0.07
Min	2.627	1.00	13.45	13.45	0.472	0.39	9.04	2.00	64.06	64.06	2.00	0.51
Max	14.79	3.50	84.95	84.85	1.78	1.47	11.32	5.00	113.05	113.05	5.00	1.28
% CV	44.59	37.23	51.47	51.44	50.94	33.38	6.98	33.33	14.80	14.80	33.33	19.53

Table 33. Summary of pharmacokinetic parameters of isoniazid plasma profile, following administration of the market FDC vs novel FDC formulations

	Formulation A (Market)						Formulation B (Novel FDC)					
	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)	$T_{1/2}$ (h)	K_{el} (h^{-1})	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)	$T_{1/2}$ (h)	K_{el} (h^{-1})
Mean	5.74	1.10	29.54	29.54	3.27	0.31	6.04	2.80	33.93	33.93	3.01	0.28
SD	2.35	0.61	11.98	11.98	2.16	0.20	0.71	0.54	4.91	4.91	1.70	0.11
SEM	0.74	0.19	3.79	3.79	0.68	0.06	0.22	0.17	1.55	1.55	0.54	0.04
Min	2.23	0.5	6.64	6.64	0.94	0.09	5.12	2	23.33	23.33	1.54	0.11
Max	8.82	2.00	39.44	39.44	7.48	0.74	7.01	3.50	39.29	39.29	6.17	0.45
% CV	40.89	55.88	40.54	40.54	65.98	65.17	11.77	19.20	14.46	14.46	56.53	39.73

The mean AUC $_{0-\infty}$ values (representing the extent of absorption) for rifampicin after administration of rifampicin and isoniazid FDC formulation, novel FDC as well as marketed FDC formulation, were found to be 92.58 µg.h/ml and 46.64 µg.h/ml respectively (Table 32). Thus, almost a 2 fold increase was observed in the AUC of rifampicin after administration of novel anti-TB FDC.

The average values of K_{el} for rifampicin were found to be 1.09 h^{-1} and 1.05 h^{-1} after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed FDC formulation, respectively. Similarly, mean $t_{1/2}$ for rifampicin after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed FDC formulation, were found to be 3.00 h and 0.77 h, respectively (Table 32). As evidenced by the respective $t_{1/2}$ values, availability of rifampicin from novel anti-TB FDC is prolonged. This is in-line with the formulation design of rifampicin formulation wherein, immediate release of rifampicin is followed by prolonged and sustained release of rifampicin.

Here it is worthwhile to mention that, for the success of the treatment of tuberculosis, good bioavailability leading to adequate plasma concentrations of rifampicin is an absolute prerequisite. It has been postulated that peak plasma rifampicin concentrations should be of the order of 10-15 µg/ml for good therapeutic response. Lower plasma levels of rifampicin results in the reduced rate of sputum conversion (Immanuel *et.al*., 2003). This further causes incomplete treatment, which may eventually leads to development of drug resistant TB (Bloomberg *et.al*., 2002).

Over the years, it has been confirmed that the bioavailability of rifampicin is significantly impaired when it is administered along with isoniazid as a FDC, in comparison with administration of formulation containing only rifampicin (Shishoo *et.al*., 2001; Immanuel, *et.al*., 2003). An almost 30% fall in bioavailability of rifampicin has been reported on administration of rifampicin isoniazid FDC. The drop of bioavailability of rifampicin from FDC products has been attributed to a facile drug-drug reaction between rifampicin and isoniazid in the acidic medium of stomach, whereby significant loss of drug occurs before absorption. The proposed mechanism demonstrates that rifampicin is first hydrolysed under acid conditions to 3-formylrifamycin, which reacts further with isoniazid to form isonicotinyl hydrazone (HYD). The HYD converts back to isoniazid and 3-FRSV, resulting in the recovery of

isoniazid, but does cause the loss of rifampicin. This explains why the bioavailability problem is confined to rifampicin alone and not to isoniazid (Shishoo *et.al*., 2001; Singh *et.al*., 2001; Singh *et.al*., 2006). This is reflected in the poor bioavailability from the rifampicin isoniazid FDC formulation. This reaction has been ascribed to be responsible for the reduced bioavailability of rifampicin from FDC products (Immanuel *et.al*., 2003; Shishoo *et.al*., 2001). The deficiency in delivery of proper dose of rifampicin has serious implications as it is known that doses of rifampicin less than 9 mg/kg body weight can result in therapeutic failure (Long *et.al*., 1979) and hence can result in the development of drug resistance.

As a result, the IUATLD and the WHO issued a joint statement advising tuberculosis control programme managers intending to use FDC drugs to purchase only products with demonstrated rifampicin bioavailability (IUTALD/ WHO, 1999).

Thus, a 2 fold increase in AUC and 18.87% increase in C_{max} of rifampicin on oral administration of novel developed FDC is an indicator of better therapeutic efficacy of rifampicin from novel anti-TB FDC.

While for isoniazid the average C_{max} values (representing the rate of absorption) after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed FDC formulation was found to be 6.04 µg/ml and 5.74 µg/ml (Table 33). The average T_{max} values for rifampicin after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed FDC formulation were 3.00 h and 2.30 h, respectively. While corresponding values of the average T_{max} for isoniazid after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed FDC formulation were 2.80 h and 1.10 h, respectively (Table 33). Delay in T_{max} value indicates a delay in absorption of isoniazid from novel anti-TB FDC. This confirms the hypothesis that the delayed release isoniazid pellets releases isoniazid at the site of maximum absorption i.e. in intestine

The average values of K_{el} for isoniazid were 0.28 h^{-1} and 0.31 h^{-1} after administration of rifampicin and isoniazid FDC formulation, novel FDC and formulation, respectively. While, mean $t_{1/2}$ for isoniazid after administration of rifampicin and isoniazid FDC

formulation, novel FDC and marketed FDC formulation, were 3.27 h and 3.01 h, respectively (Table 33).

The mean AUC $_{0-\infty}$ values (representing the extent of absorption) for isoniazid after administration of rifampicin and isoniazid FDC formulation, novel FDC as well as marketed FDC formulation, are 33.93 µg.h/ml and 29.54 µg.h/ml respectively (Table 33). Thus, it can be concluded that the extent of absorption is not affected by the delay in absorption of isoniazid from novel anti-TB FDC. This may be attributed to the fact that the HYD formed due to interaction between rifampicin and isoniazid gets converted back to isoniazid and 3-formylrifamycin, resulting in recovery of isoniazid, but eventually causing the loss of rifampicin. Thus, not affecting the extent of absorption i.e. bioavailability of isoniazid (Shishoo *et.al*., 2001; Singh *et.al*., 2001; Singh *et.al*., 2006).

Statistical analysis of the human bioavailability data

Bioavailability parameters (C_{max} and $AUC_{0-\infty}$) for both rifampicin and isoniazid from novel FDC formulation of rifampicin and isoniazid FDC were compared with that of market FDC formulation of rifampicin and isoniazid FDC by applying ANOVA test. A summary statistics of rifampicin and isoniazid on administration of rifampicin isoniazid FDC to healthy male subjects under fasting conditions are given Table 34.

The current USFDA criteria for average bioequivalence of the dosage forms requires that the mean pharmacokinetic parameters (C_{max} and $AUC_{0-\infty}$) for novel rifampicin and isoniazid FDC) should be within 80-125% of the marketed FDC formulation) using the 90% CI.

In the present study for rifampicin, it was observed that the mean pharmacokinetic parameters (C_{max} and $AUC_{0-\infty}$) did not fall within 90% CI (Table 34). Thus, the results clearly demonstrate that the two product compared are not bioequivalent. The average C_{max} and AUC $_{0-\infty}$ indicates that there is significant improvement in bioavailability of rifampicin from reference formulation as compared to that of novel FDC formulation of rifampicin and isoniazid, which is an indicator of its better therapeutic effectiveness. This is in confirmation to earlier observation made by Shishoo *et.al*., 2001 and Singh *et.al*., 2001, that segregated release of rifampicin and isoniazid in different parts of GIT tract, will prevent the two drugs coming into contact with each other in the stomach and thereby improving the bioavailability of rifampicin.

In case of isoniazid, it was observed that the mean pharmacokinetic parameters (C_{max} and $AUC_{0-\infty}$) for novel rifampicin isoniazid FDC dosage form were well within 80-125%, indicating that the two products were bioequivalent. . This is in line with the fact stated in earlier independent findings by Shishoo *et.al*., 2001 and Singh *et.al*., 2001, that the bioavailability problem is confined to rifampicin alone and not isoniazid. This has been attributed to the fact that HYD formed due to a facile interaction between rifampicin and isoniazid, is converted back to isoniazid and 3-formylrifamycin, resulting in recovery of isoniazid.

On the other hand, T_{max} value of 2.80 ± 0.17 clearly indicates a delay of approximately 3h in absorption of isoniazid (Table 34). This is in agreement with the formulation design, wherein the isoniazid is formulated in a delayed release formulation. Thus the bioavailability study provides the direct confirmation to the concept that segregated delivery of rifampicin and isoniazid from anti-TB FDC should result in improved bioavailability of rifampicin from FDC's.

Table 34. Summary statistics of rifampicin and isoniazid on administration of rifampicin isoniazid FDC to healthy male subjects under fasting conditions

	RIFAMPICIN		ISONIAZID	
Parameters	**C_{max} (µg/ml)**	**AUC_{∞} (µg.hr/ml)**	**C_{max} (µg/ml)**	**AUC_{∞} (µg.hr/ml)**
Mean				
Novel FDC (B)	10.27	92.58	6.04	33.93
Market FDC (A)	8.64	46.63	5.74	29.54
Least Square Mean (LSM)				
Novel FDC	10.243	91.58	6.00624	33.57
Market FDC	7.721	39.7671	5.12	25.86
LSM Ratio				
B/A %	132.66	230.34	117.31	129.82
90 % Confidence Interval				
Lower Limit	**88.88**	**137.92**	**100.34**	**105.548**
Upper Limit	**230.88**	**384.5**	**114.34**	**116.649**
Point estimate	1.18	1.985	1.05331	1.1486

4.5 Conclusions

The novel rifampicin isoniazid FDC capsules was prepared and evaluated for:

a. Stability studies
b. Human bioavailability studies

Based on the study the following conclusions could be drawn-

- The stability study of rifampicin-isoniazid FDC capsules was carried out at room temperature and at the accelerated stability conditions. The drug content within pharmacopoeial limits and constant water uptake indicates non significant influence of higher humidity and temperature on the novel anti-TB FDC.

- The samples of 6 M of novel FDC showed a release of 85.2 ± 1.78% of rifampicin and formation of 6.7 ± 1.12% in 60 of 3-FRSV along with 85% of release of isoniazid. The amount of 3-FRSV formed from the novel anti-TB FDC was almost 37.22% less than that of the amount formed from the marketed sample. The similarity factor, f_2 factor, of all the release profiles for the stability samples with respect to their initial values was > 50, indicative that release of rifampicin and isoniazid from the novel developed rifampicin isoniazid FDC is similar and is not affected by the higher humidity and temperature conditions. Minimal decomposition *in vitro,* thus provide a proof of concept that formulating FDC rifampicin and isoniazid with segregated site of drug delivery results in improved stability of rifampicin in the FDC.

- The novel rifampicin- isoniazid was evaluated for bioavailability of rifampicin and isoniazid in comparison to the marketed rifampicin-isoniazid FDC sample. The 90% CI for the pharmacokinetic parameter C_{max} and $AUC_{0-\infty}$ of rifampicin was well outside the bioequivalence criteria. This indicates that the two products compared are not bioequivalent. However, the average C_{max} values for rifampicin after administration of rifampicin and isoniazid FDC formulation, novel FDC and marketed formulation, were 10.27µg/ml and 8.64 µg/ml, corresponding to an increase of about 18.87%. Thus, a 2 fold increase in AUC and 18.87% increase in C_{max} of rifampicin on oral administration of novel developed FDC is an indicator of better therapeutic efficacy of rifampicin from novel anti-TB FDC.

- Also, as evidenced by the respective $t_{1/2}$ values, novel rifampicin and isoniazid FDC offers an immediate release of rifampicin followed by prolonged and the sustained release of rifampicin. This is in agreement with the formulation design of rifampicin

formulation wherein, immediate release of rifampicin is followed by prolonged and sustained release of rifampicin.

- The 90% CI for the pharmacokinetic parameter C_{max} and $AUC_{0-\infty}$ of isoniazid was well within the bioequivalence criteria. This indicates that the two products compared are bioequivalent. However, T_{max} value of 2.80 ± 0.17 clearly indicates a delay of approximately 3h in absorption of isoniazid. This indicates that the extent of absorption is not affected by the delay in absorption of isoniazid from novel anti-TB FDC. This is in agreement with the formulation design, wherein the isoniazid is formulated in a delayed release formulation.
- The bioavailability study provides the confirmation to the concept that segregated delivery of rifampicin and isoniazid from anti-TB FDC will result in improvement of bioavailability of rifampicin from FDC's without affecting the bioavailability of isoniazid. Such formulations would rule out any possibility of performance failure of the formulations due to stability-related problems during distribution and handling and especially in the climatic region IV countries.
- Finally, it can be concluded that the developed novel FDC formulation is a stable formulation with improved bioavailability. Thus, this formulation could have better therapeutic efficacy.

References

Agrawal, S., Kaur, K.J., Singh, I., Bhade, S.R., Kaul, C.L., Panchagnula, R., 2002. Assessment of bioequivalence of rifampicin, isoniazid and pyrazinamide in a four drug fixed dose combination with separate formulations at the same dose levels. Int. J. Pharm. 233, 169–177.

Bhutani, H., Mariappan, T.T., Singh, S., 2004. A study on the physical and chemical stability of anti-tuberculosis fixed-dose combination (FDC) products under accelerated climatic conditions. Int. J. Tuber. Lung Dis. 8, 1073-1080.

Blomberg, B., Evans, P., Phanouvong, S., Nunn, P., 2002. Informal consultation on 4-drug fixed-dose combinations (4FDCs) compliant with the WHO model list of essential drugs. Geneva: World Health Organization. WHO/CDS/TB/2002.299.

ICH, 2003. Stability testing of the new drug substances and products, Q1A(R2). Geneva, Switzerland.

Immanuel, C., Gurumurthy, P., Ramachandran, G., Venkatesan, P., Chandrasekaran, V., Prabhakar, P., 2003. Bioavailability of Rifampicin following concomitant administration of Ethambutol or Isoniazid or Pyrazinamide or a combination of the three drugs. Indian J. Med. Res. 118, 109-114.

IUTALD/WHO, 1994. The promise and reality of fixed dose combinations with rifampicin. A joint statement of international union against Tuberculosis and Lung diseases and tuberculosis programme of the WHO. Tuber. Lung Dis. 75, 180-181.

IUTALD/ WHO A joint statement, 1999. Assuring bioavailability of fixed-dose combinations of anti-tuberculosis medications. Int. J. Tuber. Lung Dis. 3(11), S282–S283.

Laserson, K.F., Kenyon, A.S., Kenyon, T.A., Layloff, T., Binkin, N.J., 2001. Substandard tuberculosis drugs on the global market and their simple detection. Int. J. Tuber. Lung Dis. 5, 448-454.

Long, M.W., Snider, D.R., Farer, L.S., 1979. US Public Health Service Cooperative Trial of Three RIF INH Regimens in the treatment of pulmonary tuberculosis. Am. Rev. Respir. Dis. 1979, 119, 879–894.

Luyen, L.T., Huyen, H.T.K., Sang, T.V., Huong, N.T.L., 2005. Effects of Isoniazid and Pyrazinamide on bioavailability of Rifampicin when co-administered Rifampicin-Isoniazid-Pyrazinamide. TCNCYH. 36, 12-17.

Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of Rifampicin and Isoniazid (alone and their combination) in the rat. Int. J. Tuber. Lung Dis. 7, 797-803.

Panchagnula, R., Kaur, K.J., Singh, I., Kaul, C.L., 1999. The WHO simplified study protocol in practice: investigation of combined formulations supplied by the WHO. Int. J. Tuber. Lung Dis. 3, S336– S342.

Panchagnula R., Sharma A., Agrawal S., 2003. Plasma pooling methodology as a faster and cheaper tool to evaluate bioequivalence of rifampicin component of FDCs of antitubercular drugs. Pharm. Res. 48, 655–663.

Panchagnula, R., Parmar, J., Kaur, K., Singh, I., Bade, S.R., Ashokraj, Y., 2006. Statistical evaluation of physiological variability of rifampicin in fixed dose combinations. Int. J. Pharm. 313, 5–13.

Pund, S., 2010. Formulation and Characterization of Fixed Dose Combination of Rifampicin and Isoniazid – A novel approach to improve stability and bioavailability. Ph.D. Thesis, Nirma University, Ahmedabad.

Pargal A., and Rani S., 2001. Non- linear Pharmacokinetics of rifampicin in healthy asian Indian volunteers. Int. J. Tuber. Lung Dis. 5 (1): 70-79.

Savale, S.S., 2003. Dissolution study and its correlation with bioavailability of the drugs. Ph.D. Thesis, Gujarat University, Ahmedabad.

Singh, S., Bhutani, H., Mariappan, T.T., 2006. Quality problems of anti-tuberculosis fixed-dose combinations (FDCs): A way forward. Indian J. Tuberc. 53, 201-205.

Singh, S., Mariappan, T.T., Sharda, N., Kumar, S., Chakraborti, A.K., 2000. The reason for an increase in decomposition of Rifampicin in the presence of Isoniazid under acid conditions. Pharm. Pharmacol. Commun. 6, 405- 410.

Singh, S., Mohan, B., 2003. A pilot stability study on anti-tuberculosis four drug fixed dose combination products. Int. J. Tuber. Lung Dis. 7, 298-303.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Kotecha, J.S., Shah, P.B., 1999. Stability of rifampicin in dissolution medium in presence of isoniazid. Int. J. Pharm. 190, 109-23.

Shishoo C. J., Shah S.A., Rathod I.S., and Savale S.S., 2001. Impaired bioavailability of rifampicin from fixed dose combination (FDC) formulations with Isoniazid. Indian J. Pharm. Sci. 63 (6), 443-449.

Singh S., Mariapan T. T., Sharda N., Kumar S., and Chakraborti A. K., 2000. The reason for an increse in decomposition of rifampicin in the presence of Isoniazid under acid conditions. Pharm. Pharmacol.6, 405-410.

Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.

Singh, S., Bhutani, H., Mariappan, T.T., 2006. Quality problems of anti-tuberculosis fixed-dose combinations (FDCs): A way forward. Indian J. Tuberc. 53, 201-205.

WMA (World medical association), 2008. Declaration of Helsinki Ethical principles for medical research involving human subjects. 59th WMA General Assembly, Seoul.

Chapter 5

Summary and Conclusions

"The present contains nothing more than the past & what is found in the effect was already in the cause"

..... Henri Bergson

5

Summary and Conclusions

Pharmaceutical oral solid dosage forms have been used widely for decades mainly due to their convenience of administration and their suitability for delivery of drugs for systemic effects. Among the oral solid dosage forms, controlled release drug delivery system is advantageous over conventional multidose delivery systems, particularly for long-term therapeutic effect and for the treatment of chronic diseases like TB.

TB is a major health problem in the developing countries like India, which has the maximum pool of TB patients. As of now the only available treatment lies in the effective utilization of the available anti-TB drugs. Combination of drugs can effectively counter this problem that led to the concept of FDCs. However, the bioavailability and stability of rifampicin in anti-TB FDC has come as a major 'bottleneck' in the treatment of TB. Rifampicin is the only sterilizing drug and a critical component in the therapeutic armamentarium for TB. 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 the doses of rifampicin less than 9mg/kg body weight can result in therapeutic failure and hence can be the cause of development of drug resistance. The problems associated with quality of FDC products are in the current focus. During the last few years, sufficient data has been generated that indicates the interaction of rifampicin and isoniazid in the acidic medium of the stomach and in the solid dosage form.

It is thus expected that bioavailability concerns associated with rifampicin could be overcome by developing a system that attains segregated delivery of the two drugs, with rifampicin being released immediately in the stomach and isoniazid in the small intestine (through development of an enteric-release system), thus targeting them to their respective absorption windows. This strategy would also preclude physical interaction of these drugs within the dosage form during storage. In view of this, in present study the

novel formulation was designed and developed 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 rifampicin immediate release pellets were prepared using extrusion-spheronization. It was found that in contrast to other superdisintegrants, indion 414 retained its functionality even after wet extrusion under high pressure and efficiently increased the drug release from the pellets. A 3^2 full factorial experimental design was applied to optimise the rifampicin immediate release formulation. The optimised rifampicin immediate release pellets showed satisfactory micromeritic, mechanical and release profile (Usable yield > 90%, narrow pellet size distribution, % fines nil, roundness score and pellips near to 1, friability less than 1% and dissolution NLT 75% (Q) in 45 min.). The optimised rifampicin pellets were found to be stable at accelerated stability conditions (40°C ± 2°C/75% RH ± 5%) and at room temperature for 6 M (Assay 98.99-100.67%; water content 3.22-4.47% and dissolution NLT 75% (Q) in 45 min.).

The floating rifampicin tablet prepared by using a combination of HPMC K4M and Carbopol was optimised using 3^2 full factorial experimental design. The optimized formulation gave sufficient duration of floating (>520 min.) and a shorter duration of floating lag time (< 3 min.) *in vitro*. These tablets were found to have desirable physical properties (friability < 1%, drug content: 100.15% ± 2.15). The *in vivo* performance of the floating rifampicin tablet was assessed using gamma-scintigraphy technique in human volunteers. Gamma-scintigraphic studies in human volunteers reveals that rifampicin floating formulation remains in the stomach for ~6 h. T_{50} for gastric emptying from gamma-scintigraphic study of floating rifampicin formulation was found to be around 345 min. in comparison to the marketed rifampicin (immediate release) formulation having $T_{50,}$ for gastric emptying, of around 90 min. The optimised floating rifampicin tablets were also found to be stable at accelerated stability conditions and at room temperature, for 6 M.

Delayed release isoniazid pellets were prepared using extrusion-spheronization followed by coating with enteric polymer using wruster technique. The core of isoniazid pellet was optimized using 2^3 full factorial design. Isoniazid pellets coated with 30% enteric coating, were found to be completely intact at the end of 2 h in 0.1N HCl and only 9.9% of isoniazid was released in 2 h in acid resistance test. While, at pH 6.8, ~80.60% of Isoniazid was released at the end of 45 min. The optimised isoniazid delayed release pellets were found to be stable and passed the gastric acid resistance test even after subjecting them to 6 M accelerated stability conditions and also at room temperature (Assay 98-102%; water content 1.88-2.88%).The stability samples of delayed release isoniazid pellets also passed the gastric acid resistance test and released not more than 8% drug in the phosphate buffer pH 6.8.

The novel rifampicin isoniazid FDC capsules were prepared and subjected for stability studies and human bioavailability studies. The stability samples of novel FDC showed a release of 85.2 ± 1.78% of rifampicin and formation of 6.7 ± 1.12% in 60 min. of 3-FRSV along with 85% of release of isoniazid. The amount of 3-FRSV formed from novel anti-TB FDC was almost 37.22% less than that of the amount formed from the marketed sample. The drug content within pharmacopoeial limit, constant moisture content and similarity factor more than 50 indicates that the novel developed rifampicin isoniazid FDC is similar and is not affected by the higher humidity and temperature conditions. Minimal decomposition *in vitro*, thus provide a proof of concept that formulating FDC rifampicin and isoniazid with segregated site of drug delivery results in improved stability of rifampicin in the FDC.

The bioavailability of the developed novel rifampicin and isoniazid FDC in comparison to a marketed rifampicin and isoniazid FDC, was carried out in healthy human volunteers in an open label, balanced, randomised, three-treatment, three-sequence, three period, crossover, single centre bioavailability study of single oral dose of fixed dose combination of rifampicin and isoniazid. The 90% CI for the pharmacokinetic parameter C_{max} and $AUC_{0-\infty}$ of rifampicin was well outside the bioequivalence criteria indicating that the developed novel rifampicin isoniazid FDC was not bioequivalent with marketed rifampicin and isoniazid FDC. While, the 90% CI for the pharmacokinetic parameter C_{max} and $AUC_{0-\infty}$ of isoniazid was well within the bioequivalence criteria. This indicates

that the two products compared (developed FDC vs Marketed FDC) are bioequivalent for isoniazid.

However, a 2 fold increase in AUC and 18.87% increase in C_{max} was observed for rifampicin on oral administration of novel developed FDC, which indicates better therapeutic efficacy of rifampicin from novel anti-TB FDC.

Also, the plasma profile of isoniazid from the novel FDC showed a lag time of 2 h followed by a rapid absorption. However, the extent of absorption was not affected by the delay in absorption (AUC) of isoniazid from novel anti-TB FDC. This is in agreement with the formulation design, wherein the isoniazid is formulated in a delayed release formulation.

The bioavailability of rifampicin was found to be improved from the developed FDC of rifampicin and isoniazid in comparison to the marketed FDC of rifampicin and isoniazid. The bioavailability study provides the confirmation to the concept that segregated delivery of rifampicin and isoniazid from anti-TB FDC will result in improvement of bioavailability of rifampicin from FDC's without affecting the bioavailability of isoniazid. This is in accordance with the rationale of the need to redesign the FDC of rifampicin and isoniazid.

Thus, from the present study it can be **concluded** that-

- With the aim to overcome the quality problems associated with rifampicin in the currently available rifampicin isoniazid FDC, a novel anti-TB FDC was developed. In the developed novel FDC, rifampicin was retained in the stomach and isoniazid was delivered 3-4 h later in the intestine.
- An oral FDC of rifampicin and isoniazid that ensured differentiated release of rifampicin and isoniazid in the GI tract was developed, incorporating the optimum floating rifampicin tablet and enteric coated isoniazid pellet formulation.
- The developed novel rifampicin and isoniazid FDC composition represents a stable FDC formula with minimal decomposition at room temperature and accelerated stability conditions. The stability of the developed formulation indicates that this strategy would also preclude physical interaction of these drugs within the dosage

form during storage. Such formulations would rule out the possibility of failure in the performance of formulations due to stability-related problems during distribution and handling and especially in region IV countries. Region IV countries have high temperature and humidity and fall mainly in TB high-burden category countries.

- The developed novel FDC of rifampicin and isoniazid, with inbuilt segregated delivery approach of both the drugs in the GI tract, has resulted in the improved bioavailability in comparison to the marketed FDC of rifampicin and isoniazid. This establishes a proof of concept for the improved bioavailability of rifampicin from the developed novel FDC formulation.
- This developed system provides simultaneous controlled delivery of both the drugs (rifampicin and isoniazid) in a way to reduce the problem of interaction between them and improved efficacy of the FDC.

The developed novel FDC formulation of rifampicin and isoniazid has the potential for improving the effectiveness of the current anti-TB FDCs, possibly with reduced treatment period. The enhanced efficacy of the FDC can reduce the chances of emergence of drug resistance strains of mycobacterium. However, further investigations needs to be carried out in this direction.

Annexure 1

List of Instruments and Materials

List of Instruments

Column Kromasil (C18, BDS)	LCGC, USA
Design-Expert software	v. 7.1.2, Stat-Ease Inc., Minneapolis, USA
Differential Scanning Calorimeter	Perkin-Elmer-7, Perkin Elmer, USA
Extruder	R.R Enterprise, Mumbai
Fluidised bed dryer	Niro Aeromatic, Switzerland
Gamma camera	Infinia ^{TM}, GE, India
Hardness tester	ET 101, Electrolab India, Mumbai
Helium pycnometer	Smart Pycno 30, Smart Instruments, Mumbai
High performance liquid chromatography	Jasco, Japan
HPLC autosampler	Jasco AS95010, Tokyo, Japan
HPLC Solvent pump	Jasco PU 980, Tokyo, Japan
HPLC UV detector	Jasco UV 875, Jasco, Tokyo, Japan
Humidity chamber	EIE Instruments Pvt. Ltd., Ahmedabad
Image analysis software	AnalySIS, v. 5.2, Soft Imaging System, Münster, Germany
Karl Fischer titrator	Systronics Universal titrator 353, India
Malvern Mastersizer	Malvern 2000, Malvern Instruments, UK
Moisture balance	HB43-S, Mettler Toledo, Ohio, USA
Planetary mixer	Kalweka, Karnavati Eng. Ltd., India
Roche friabilator	EF-2, Electrolab, India
Scanning electron microscope	JEOL JSM 6100, JEOL, Japan
Sieve shaker	EMS-8, Electrolab, India
Single station tablet machine	Cadmach Machinery Co, Ltd, Ahmedabad, India
Spheronizer	R.R Enterprise, Mumbai
Sputter coater unit	JEOL, JFM 1100, Japan
Stereomicroscope	Leica S4E, Germany
Tablet strength tester	EH 01, Electrolab, India
Tapped density tester	LabHosp, India
Ultra centrifuge	Biofuge, Sorvall, Thermo Scientific, India
USP Dissolution apparatus I	SR8 Plus Hanson Research Corporation, Chatsworth
UV-VIS spectrophotometer	Shimadzu UV-2450, Japan

Weighing Balance	AE 240, Metler Toledo, USA

List of Materials

Ac-di-sol	Litaka Twilight, Pune
Acetonitrile	Qualigens, New Delhi, India
Acryl-eze	Colorcon India, Mumbai
Ascorbic acid	S.D Fine Chem. Ltd., Mumbai
Avicel PH 101	Signet Chem. Corporation, India
Cellulose acetate phalate	Instacoat, Mumbai
Chloroform	Qualigens, Mumbai, India
Crosspovidone	Litaka Twilight, Pune
Di sodium hydrogen orthophosphate	Qualigens, New Delhi, India
Dichloromethane,	Qualigens, New Delhi, India
Eudragit RL- 100	Degussa India, Mumbai
Eudragit® NE 30D	Evonik industries, India
HPMC K4M	Colorcon, India
Hydrochloric acid (HCl)	Rankem, New Delhi, India
Indion 414	Ion exchange, Mumbai
Isoniazid	Cadila Pharmaceuticals Limited, Ahmedabad
Lactose	Lactose India, India
Methanol	Qualigens, New Delhi, India
Methocoel A15LV	Colorcon, India
Papaverine hydrochloride	Biologicals E. Ltd, India
Poly Ethylene Glycol (PEG) 6000	Cadila Pharmaceuticals Limited, India
Polyvinyl pyrrolidone (PVP) 90	BASF, Ludwigshafen, Germany
Potassium dihydrogen orthophosphate	Qualigens, New Delhi, India
Pyrizinamide	Macleods Pharmaceuticals, India
Rifampicin	Cadila Pharmaceuticals Limited, Ahmedabad
Sodium Alginate	S.D Fine Chemicals, India
Sodium bicarbonate	S.D Fine Chemicals, India
Sodium Carboxymethyl Cellulose	S.D Fine Chemicals, India
Triethyl Citrate (TEC)	S.D Fine Chemicals, India

Patent, Publications

Presentations

Patent

- **Indian patent 2829/MUM/2009.** Oral gastroretentive Fixed dose pharmaceutical composition for the treatment of tuberculosis and process for preparation thereof.

Publications

- Pund S., **Joshi A.,** Vasu K., Nivsarkar M., and Shishoo C. J., Multivariate optimization of formulation and process variables influencing physico-mechanical characteristics of site-specific release isoniazid pellets- ***Int. J. Pharm.,*** *2010, 388, 64–72.*

- **Joshi A.,** Pund S., Nivsarkar M., Vasu K., and Shishoo C. J., Dissolution test for site-specific release isoniazid pellets in USP Apparatus 3 (reciprocating cylinder): Optimization using Response Surface Methodology. ***Eur. J. Pharm. Biopharm***. *2008, 69, (2), 769-775.*

Presentations

- **Joshi A.,** Pund S., Nivsarkar M., Vasu K., and Shishoo C. J., Functionality comparison of superdisintegrants in promoting dissolution of Rifampicin pellets"- *12th APTI Annual National Convention, Chandigarh, India, 25th-27th October, 2007.*

- Pund S., **Joshi A.,** Vasu K., Nivsarkar M., and Shishoo C. J., An application of Plackett-Burman design to study factors influencing dissolution from isoniazid pellets in USP apparatus 3 -*12th APTI Annual National Convention, 25th-27th October 2007, Chandigarh, India*.

- **Joshi A.,** Pund S., Nivsarkar M., Vasu K., and Shishoo C. J., Design and Development of a Novel Site-Specific Delivery System of Rifampicin and Isoniazid for Improved Stability and Bioavailability"- *1st Indo-Japanese International Joint Symposium on Overcoming Intractable Infectious Diseases, Tokyo, Japan, 6th-7th November 2007.*

- Pund S., **Joshi A.,** Vasu K., Nivsarkar M., and Shishoo C. J., Study of Solid-Solid Interaction of Rifampicin and Isoniazid by Differential Scanning Calorimetry- *1st Indo-Japanese international symposium on overcoming intractable diseases prevalent in Asia, 6th and 7th November 2007, Tokyo, Japan.*

Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 769–775
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Dissolution test for site-specific release isoniazid pellets in USP apparatus 3 (reciprocating cylinder): Optimization using response surface methodology

Amita Joshi, Swati Pund, Manish Nivsarkar, Kamala Vasu, Chamanlal Shishoo *

B. V. Patel PERD Centre, Department of Pharmaceutics, Ahmedabad, India

Received 11 September 2007; accepted in revised form 30 November 2007
Available online 14 December 2007

Abstract

The present work aims to predict drug release from novel site-specific release isoniazid pellets, in USP dissolution test apparatus 3, using the response surface methodology (RSM). Site-specific release isoniazid pellets were prepared by extrusion-spheronization followed by aqueous coating of Acryl-EZE®. RSM was employed for designing of the experiment, generation of mathematical models and optimization study. A 3^2 full factorial design was used to study the effect of two factors (at three levels), namely volume of dissolution medium (150, 200, 250 ml) and reciprocation rate (5, 15, 25 dips per min). Amount of drug released in 0.1 N hydrochloric acid at 2 h and in pH 6.8 phosphate buffer at 45 min were selected as responses. Results revealed that both, the volume of medium and reciprocation rate, are significant factors affecting isoniazid release. A second order polynomial equation fitted to the data was used to predict the responses in the optimal region. The optimized conditions resulted in dissolution data that were close to the predicted values. The proposed mathematical model is found to be robust and accurate for optimization of dissolution test conditions for site-specific release isoniazid pellets.

Keywords: Isoniazid; Site-specific release; USP apparatus 3; Response surface methodology; Optimization; Full factorial design

1. Introduction

Isoniazid, an isonicotinic acid hydrazide, a first-line antitubercular agent, is an integral part of intensive as well as continuation phase of six months treatment schedule against tuberculosis [1]. Isoniazid has an aqueous solubility of approximately 125 mg ml^{-1} [2]. In order to minimize its interaction with rifampicin in acidic environment of stomach, Shishoo et al. [3] emphasized the need to develop a site-specific release formulation of isoniazid. Isoniazid is less permeated through the stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH ($pK_a = 2$) [4]. Therefore, it can be considered as a good candidate for the development of a site-specific release formulation. Enteric coating is a popular and a widely accepted technique for achieving the site-specific drug release in the intestine. Considering the popularity and the robustness of the multiparticulate system (e.g., pellets, granules, etc.) as a means of tailoring the release profile of a drug [5], this approach has been adopted in the formulation of isoniazid pellets. Pellets offer various advantages over single unit dosage form including minimal risk of dose dumping, flexibility of blending units with different release patterns, as well as short and reproducible gastric residence time [6].

Dissolution test has proved to be an essential *in vitro* test to characterize the performance of an oral drug delivery system [7]. The significance of a dissolution test is that, for a drug to be absorbed from gastrointestinal tract and to be available to the systemic circulation, it must be previously solubilized [8]. Therefore, dissolution test is used not

* Corresponding author. B. V. Patel PERD Centre, Department of Pharmaceutics, Sarkhej-Gandhinagar Highway, Thaltej, Ahmedabad 380054, India. Tel.: +91 79 27439375; fax: +91 79 27450449.
E-mail address: perd@perdcentre.com (C. Shishoo).

0939-6411/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2007.11.020

only for the quality control of a finished product to assess batch-to-batch consistency of drug release from solid dosage forms, but is also essential in the development of a formulation for screening and proper assessment of different formulations. In fact, the creative use of dissolution technique can speed up the formulation development, particularly in the case of modified-release products, enabling prompt identification of potential problems in drug release rate. Essentially, dissolution test makes it possible to assess the dissolution properties of the drug itself and thereby to select the most appropriate excipients and appropriate proportions among them for obtaining the desired drug release behavior. Among the several dissolution methods specified in United States Pharmacopoeia (USP), apparatus 1 (basket) has been extensively employed to evaluate the dissolution of site-specific release formulations. However, USP apparatus 3 (reciprocating cylinder) provides sound hydrodynamic conditions for the evaluation of pellets. In contrast to the movement of media in USP apparatus 1, the dosage form moves freely through the dissolution medium in case of reciprocating cylinder. USP apparatus 3 is considered as the first line apparatus in product development of controlled release products and especially the pellets, because of its usefulness and convenience in exposing products to mechanical as well as variety of physicochemical conditions which eventually influence the release of a product in the gastrointestinal tract. USP apparatus 3 has a relatively short history and was incorporated into USP in 1991 as apparatus 3 [9]. There exist only a few reports in the literature on the use of USP apparatus 3 for testing drug release rate and for comparing it to those obtained from other methods. Most of these reports, however, focus on extended release dosage forms [10–13].

Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery systems [14,15]. Based on the principle of design of experiments, the methodology encompasses use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to optimize formulation as well as processing conditions. The advantage of such methodology is in providing a rationale for simultaneous evaluation of several variables. The technique requires minimum experimentation and time, thus proving to be far more efficient and cost effective than conventional methods of product development. For implementation of RSM, factorial designs (FDs; full or fractional) are the most popular statistical designs. Full factorial design (FFD) involves the study of the effect of all factors at various levels and is considered as an efficient approach to estimate the influence of individual variables (main effect) and their interactions. Until date, application of RSM has not been reported in the development and optimization of the dissolution test method for USP apparatus 3. Most of the publications however focus on the optimization of dissolution test conditions for USP apparatus 1 and 2 [14,16].

The current study illustrates the evaluation of *in vitro* release characteristics of site-specific release isoniazid pellets, under defined hydrodynamic conditions in USP apparatus 3. Computer-aided optimization techniques using 3^2 FFD were employed to investigate the effect of two factors viz., volume of the dissolution medium and reciprocation rate, on release of isoniazid from the site-specific release pellets. A FD for 2 factors at 3 levels each (3^2) is considered identical to a two-factor composite design and has an added advantage of determining a quadratic response surface [14,15,17].

2. Materials and methods

2.1. Materials

Isoniazid I. P. was kindly supplied by Cadila Pharmaceuticals Ltd., Ahmedabad, India. Microcrystalline cellulose (Avicel® PH 101, Signet Chemical Corporation, Mumbai, India), Polyvinylpyrrolidone (PVP K-90, Kollidon® 90, BASF, Germany) and Acryl-EZE® (Colorcon Asia Pvt. Ltd., Mumbai, India) were used as excipients and were obtained from the indicated sources. All other ingredients and reagents were of analytical grade and were used as received.

2.2. Preparation of site-specific release isoniazid pellets

2.2.1. Preparation of isoniazid loaded pellets

Powder components of the formulation (Isoniazid – 55% w/w, Avicel® PH 101 – 42% w/w and Kollidon® 90 – 3% w/w; Batch size – 500 g) were mixed in a small scale planetary mixer (Kalweka, Karnavati Eng. Ltd., India) for 10 min. Purified water (40% w/w of total solids) was added to get a wet mass. Extrudates were obtained by feeding the wet mass in gravity fed cylinder extruder (R. R. Enterprises, India). Extrudates were spheronized in a spheronizer (R. R. Enterprises, India) to obtain spherical pellets. The pellets were dried in fluid-bed dryer (Nero-Aeromatic, Switzerland) at 50 °C for 20 min. Fraction of pellets, 16/25#, was subjected to coating process.

2.2.2. Enteric coating of isoniazid pellets for site-specific release of isoniazid

Isoniazid pellets were coated with 10% w/w aqueous suspension of Acryl-EZE® using fluid-bed coater (Nero-Aeromatic, Switzerland) to achieve 35% weight gain. The process conditions were 'pre-warming of the cores at 40 °C for 10 min; spray nozzle diameter, 1 mm; atomizing air pressure, 1 bar; air flow rate, 80 $m^3\ h^{-1}$; inlet air temperature, 40 °C; product temperature 32–35 °C; spray rate, 1.5 ml min^{-1}; post drying at 40 °C for 10 min.

2.3. Dissolution methodology

Dissolution studies were carried out in USP dissolution apparatus 3 (Hanson Research B-3 release rate tester;

Hanson Research Corporation, Chatsworth, CA). For carrying out release rate study, USP method B, for delayed release formulations, was followed [18]. Test was carried out in 0.1 N hydrochloric acid (HCl) for 2 h followed by pH 6.8 phosphate buffer USP for 45 min. Dissolution medium, pH 6.8 phosphate buffer were prepared by combining appropriate amounts of HCl and tri-basic sodium phosphate. Table 1 summarizes the general conditions followed in this study. During the study, the reciprocating cylinder containing pellets moved between the rows successively and switched from one medium to another.

All the dissolution samples were filtered through 0.22 μm Millipore® (Polyvinylidene difluoride, PVDF) filter and analyzed immediately after the completion of dissolution test by UV-Visible spectrophotometer (Shimadzu UV-2450, UV-vis scanning spectrophotometer, Japan). Isoniazid released in 0.1 N HCl was estimated as per method specified in USP [19] and isoniazid released in pH 6.8 phosphate buffer was measured at λ_{max} 263 nm by a validated spectrophotometric method [20]. The analytical method was found to be specific, linear in the concentration range of 5–30 μg/ml, precise (%CV: 1.05–3.16) and accurate (98.5–102.0%). For each dissolution run, a mean of six determinations was recorded.

2.4. Experimental design

A 3^2 FFD was used for the dissolution testing optimization procedure. Volume of dissolution medium (X_1, ml) and reciprocation rate (X_2, dips per minute, dpm) were the two factors (independent variables) studied. The levels for X_1 and X_2 were chosen in accordance with the preliminary data and were representative of the entire range of operating conditions of the USP apparatus 3. The responses (dependent variables) studied were amount of isoniazid released in 0.1 N HCl at 2 h (Y_1, %) and amount of isoniazid released in pH 6.8 phosphate buffer at 45 min (Y_2, %). Table 2 summarizes independent and dependent variables along with their levels. Experimental dissolution testing runs are listed in Table 3.

Table 1
The test conditions followed in the dissolution testing of site-specific release isoniazid pellets in USP apparatus 3

Parameter	Dissolution test conditions
Dissolution medium	0.1 N HCl and pH 6.8 phosphate buffer USP
Temperature (°C)	37.0 ± 0.5
Volume (ml)	150/200/250 (as per the statistical design)
Reciprocation speed (dpm)	5/15/25 (as per the statistical design)
Sample holder	Reciprocating cylinder (#40)
Volume of the sample withdrawn (ml)	5.00
Test duration	2 h in 0.1 N HCl followed by 45 min in pH 6.8 phosphate buffer

Table 2
Factors (independent variables), factor levels and responses (dependent variables) used in 3^2 full factorial experimental design

Factors	Factor levels used			Responses
	−1	0	1	
X_1 = Volume of dissolution medium (ml)	150	200	250	Y_1 = amount of isoniazid released in 0.1 N HCl at 2 h (%) and
X_2 = Reciprocation rate (dpm)	5	15	25	Y_2 = amount of amount released in pH 6.8 phosphate buffer at 45 min (%)

Table 3
Dissolution test runs carried out on site-specific release isoniazid pellets as per 3^2 full factorial experimental design

Dissolution test run	Factor X_1 (Volume of dissolution medium, ml)	Factor X_2 (Reciprocation rate, dpm)
1	150	5
2	200	5
3	250	5
4	150	15
5	200	15
6	250	15
7	150	25
8	200	25
9	250	25

2.5. Statistical analysis of the data and validation of the model

Various RSM computations for the current study were performed employing Design-Expert® software (Version 7.1.2, Stat-Ease Inc., Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. Statistical validity of the polynomials was established on the basis of ANOVA and the 3D response graphs were constructed using Design-Expert® software. To validate the chosen experimental design and polynomial equations, optimum test condition was selected. The tests corresponding to this optimum dissolution condition and three additional random dissolution test conditions were carried out in the experimental matrix to determine the validity of the model generated. Subsequently, the resultant experimental data of the response properties were quantitatively compared with those of the predicted values. Also, the linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel.

3. Results and discussion

In developing a novel drug delivery system, particularly, in the case of site-specific release product, dissolution test is a helpful *in vitro* tool for the assessment and adjustment of the drug release profile from a candidate formulation, enabling easy and fast evaluation of the effects of formulation changes. However, this test is sensitive to many

parameters such as temperature, agitation, dissolution medium, pH of the medium, volume of dissolution medium and shape of the vessel [21,22]. The precision of dissolution test is essential for the reliability of the results. Earlier experiments in our laboratory using USP dissolution apparatus 1 indicated a non discriminatory dissolution test (data not shown). Therefore, USP dissolution apparatus 3 was chosen for the current study. A multivariate optimization strategy was carried out with the aim of finding the optimum conditions for the testing of drug dissolution behavior from the site-specific release isoniazid pellets.

3.1. Experiments of 3^2 FFD

Response data for all the 9 experimental runs of 3^2 FFD, performed in accordance with Table 3, are presented in Table 4. In 0.1 N HCl only 11.40% to 15.90% of isoniazid was released in 2 h and pellets were found to be completely intact at the end of 2 h. Acid resistance test is a significant index of drug dissolution performance of an enteric coated formulation. Polymers used for formulating enteric coated formulation should be able to withstand the lower pH values of stomach and be able to disintegrate in the range of pH 6–7. At pH 6.8, 67.90% to 80.60% of isoniazid was released at the end of 45 min. This indicates that Acryl-EZE® effectively controls the release of isoniazid (a borderline BCS class-I and class-III drug, [23]) from site-specific release isoniazid pellets.

3.2. Mathematical modeling

Mathematical relationship was generated between the factors (dependent variables) and responses (independent variables) using the statistical package Design-Expert® for determining the levels of factors, which yield optimum dissolution responses. A second order polynomial regression equation that fitted to the data is as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2, \quad (1)$$

where β_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; β_1 to β_5 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the coded levels of factors. The terms X_1X_2 and X_i^2 ($i = 1$ and 2) represent the interaction and quadratic terms, respectively. The equations of the responses are given below:

$$Y_1 = 11.52 - 0.58X_1 + 0.01X_2 + 0.58X_1X_2 + 2.82{X_1}^2 + 0.47{X_1}^2 \quad (2)$$

$$Y_2 = 70.72 + 1.88X_1 + 3.38X_2 + 4.9X_1X_2 + 0.88{X_2}^2 - 1.32{X_2}^2 \quad (3)$$

The equation represents the quantitative effect of factors (X_1 and X_2) upon the responses (Y_1 and Y_2). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors.

Analysis of variance (ANOVA) was applied for estimating the significance of the model, at 5% significance level. A model is considered significant if the p-value (significance probability value) is less than 0.05. From the p-values presented in Table 5, it can be stated that for both the responses the linear contribution of the model was not significant. However, for response Y_1, quadratic contribution of the response was significant, whereas, for response Y_2, the cross product contribution was significant.

In Table 6, factor effects of 3^2 FFD model and associated p-values for the responses Y_1 and Y_2, are presented. A factor is considered to influence the response if the effects significantly differ from zero and the p-value is less than 0.05.

Data in Table 6 show that the response Y_1 was significantly affected by the synergistic effect of quadratic term

Table 4
Results of dissolution studies carried out on site-specific release isoniazid pellets as per 3^2 full factorial experimental design: response Y_1 (amount of isoniazid released in 0.1 N HCl at 2 h, %) and response Y_2 (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min, %)

Dissolution test run	Response Y_1 (%)[a]	Response Y_2 (%)[a]
1	15.9 ± 0.7	68.9 ± 3.6
2	15.3 ± 1.5	70.6 ± 3.6
3	14.5 ± 1.2	67.9 ± 4.2
4	11.4 ± 0.9	69.8 ± 1.7
5	11.6 ± 0.7	68.1 ± 4.5
6	12.5 ± 1.2	72.5 ± 3.1
7	14.0 ± 0.5	62.0 ± 4.5
8	13.3 ± 0.8	76.1 ± 2.1
9	14.9 ± 0.5	80.6 ± 2.3

[a] Mean of 6 ± SD.

Table 5
Summary of analysis of variance (ANOVA) for the measured response Y_1 (amount of drug released in 0.1 N HCl at 2 h) and response Y_2 (amount of drug released in pH 6.8 phosphate buffer at 45 min)

Source of variation	Y_1		Y_2	
	F	p-value	F	p-value
Linear contribution	0.34	0.7273	2.00	0.2157
Quadratic contribution	10.36	0.0413	3.40	0.1714
Cross-product contribution (2FI)	0.33	0.8067	8.00	0.0235

Table 6
A summary of each factor effect and its p-values for, response Y_1 (amount of isoniazid released in 0.1 N HCl at 2 h) and for response Y_2 (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min)

Factor	Y_1		Y_2	
	Factor effect	p-value	Factor effect	p-value
X_1	−0.580	0.1035	+1.880	0.1584
X_2	+0.100	0.7179	+3.380	**0.0309**
X_1X_2	+0.580	0.1592	+4.900	**0.0169**
${X_1}^2$	+2.820	**0.0075**	+0.880	0.7342
${X_2}^2$	+0.432	0.3632	−1.320	0.6174

Significant effects of factors on individual responses are shown in bold type.

of volume of dissolution medium (X_1^2) (p-value, 0.0075). Significant factors affecting the response Y_2 were reciprocation rate (X_2) with p-value, 0.0309 and interaction effects (cross-product terms) with p-value, 0.0169. Both the above-mentioned factors show the synergistic effect and increase the release of isoniazid from site-specific release isoniazid pellets.

3.3. Response surface analysis

The 3-dimensional response surface plots were drawn to estimate the effect of independent variables on each response. Figs. 1 and 2 show the effect of two hydrodynamic conditions in the dissolution test on the release of isoniazid in 0.1 N HCl and release of isoniazid in pH 6.8,

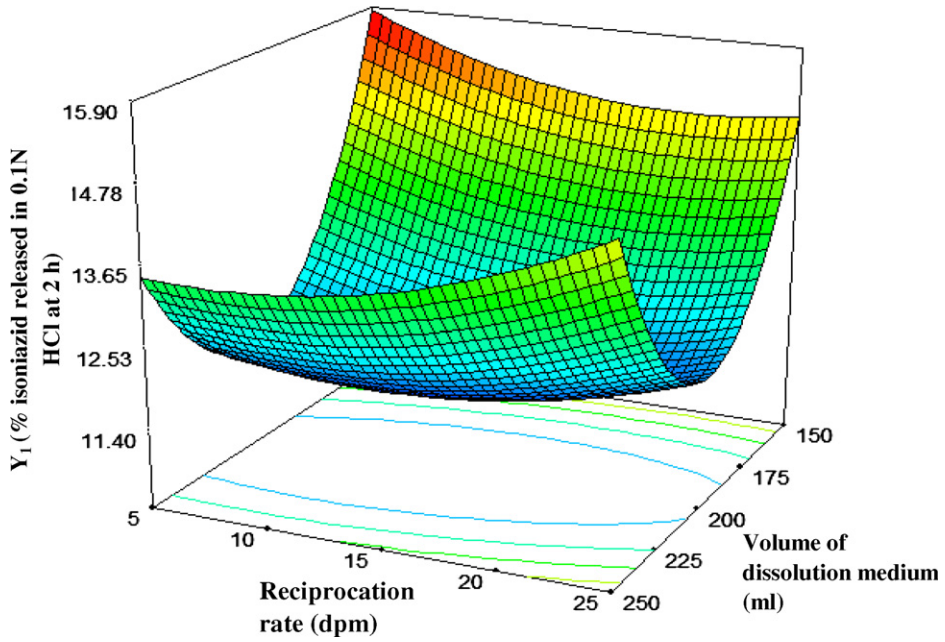


Fig. 1. Response surface plot showing the influence of volume of dissolution medium and reciprocation rate on response Y_1 (amount of isoniazid released in 0.1 N HCl at 2 h, %).

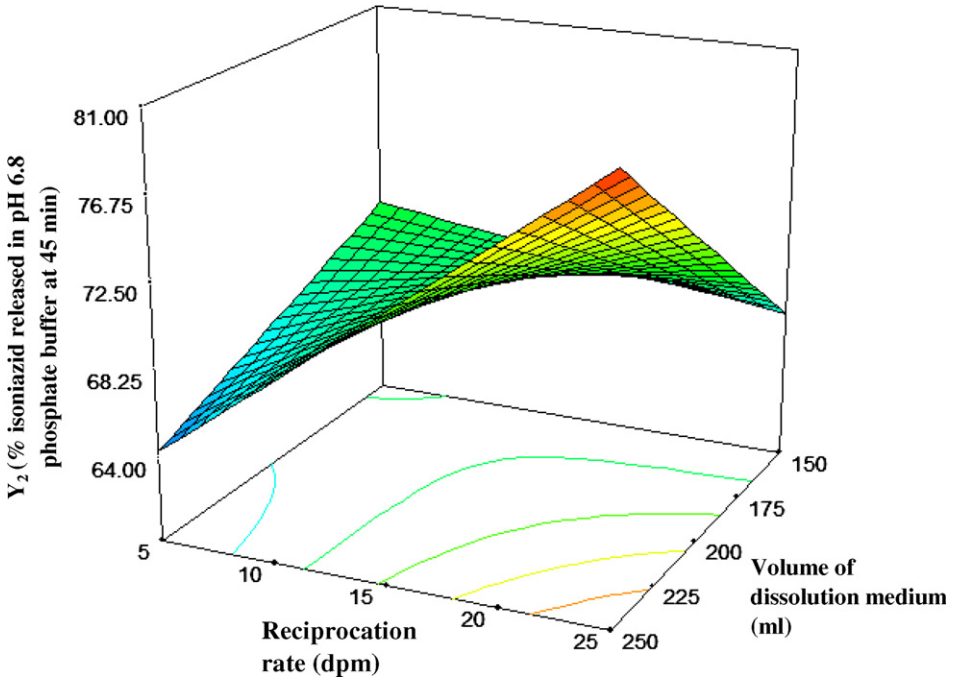


Fig. 2. Response surface plot showing the influence of volume of dissolution medium and reciprocation rate on response Y_2 (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min, %).

respectively. The Fig. 1 shows 'a region of minima' lying between the intermediate to higher levels of both the factors. However, the effect of volume of dissolution medium seems to be more pronounced as compared with that of speed. This receives confirmation from the mathematical model generated for response (Eq. 2).

Fig. 2 depicts a nonlinear twisted relationship for Y_2 at intermediate and high levels of both the factors. This can be attributed to the potential occurrence of interaction between the two independent variables at the corresponding factor levels, construing that each independent variable is tending to modify the effect of another towards the release of isoniazid in pH 6.8. However, the effect of speed seems to be more pronounced as compared with that of volume of dissolution medium. This is in agreement with Eq. (3) as well as Fig. 2.

With the help of polynomial equation, the process was optimized for both the responses. The final optimal experimental parameters were calculated by satisfying the requirements for each response in the set. Thus, to obtain site-specific release of isoniazid, it is desirable to minimize Y_1, and maximize Y_2. In this study optimization was performed with constraints for Y_1 ($\leqslant$15%) and Y_2 ($\geqslant$ 80%). The optimal calculated parameters were

- Volume of dissolution medium, $X_1 = 225$ ml
- Reciprocation rate, $X_2 = 25$ dpm

The test carried out with the above-mentioned dissolution test conditions showed $Y_{1\text{Experimental}}$ as 12.00% ($Y_{1\text{Predicted}}$, 12.31%; percentage prediction error, −2.58) and $Y_{2\text{Experimental}}$ as 82.63% ($Y_{2\text{predicted}}$, 80.6%; percentage prediction error, 2.46) as shown in Table 7. Low values of prediction percentage error indicate that the predicted and observed values are in good agreement.

3.4. Validation of response surface model

In order to assess the reliability of the developed mathematical model, dissolution tests corresponding to the above-mentioned optimum dissolution conditions and three additional random dissolution tests with conditions covering the entire range of experimental domain were performed. For each of these test runs, responses were estimated by use of the generated mathematical model and by the experimental procedures. Table 7 lists the dissolution test conditions of the optimum and the random check points, their experimental and predicted values for both the response variables. Fig. 3A and B shows linear correlation plots between the observed and predicted response variables. The graphs demonstrate high values of correlation coefficient, r^2 (>0.9) indicating excellent goodness of fit. Therefore, it can be concluded that, model functions Y_1

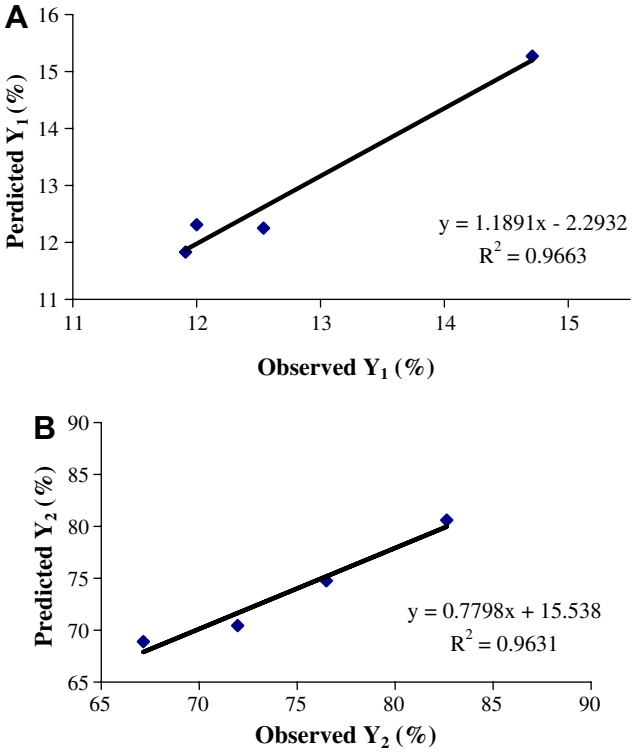


Fig. 3. Linear correlation plots (A and B) between observed and predicted values for response Y_1 (amount of isoniazid released in 0.1 N HCl at 2 h,%) and response Y_2 (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min, %).

Table 7
The experimental and predicted values for response Y_1 (amount of isoniazid released in 0.1 N HCl at 2 h, %) and for response Y_2 (amount of isoniazid released in pH 6.8 phosphate buffer at 45 min, %) along with percentage prediction error observed for the optimum dissolution test condition (A) and random dissolution test conditions (B, C, and D)

Dissolution test	Test conditions[a] X_1 (ml)/X_2 (dpm)	Response	Experimental value	Predicted value	Percent prediction error[b]
A	225/25	Y_1	12.00	12.31	−2.58
		Y_2	82.63	80.6	2.46
B	200/10	Y_1	11.91	11.83	0.67
		Y_2	67.15	68.9	−2.60
C	225/20	Y_1	12.54	12.25	2.31
		Y_2	76.49	74.76	2.26
D	150/10	Y_1	14.71	15.27	−3.80
		Y_{21}	71.96	70.44	2.11

[a] X_1, volume of the dissolution medium (ml) and X_2, reciprocation rate (dpm).
[b] Percent error was calculated using the formula (Experimental value − Predicted value)/Experimental value × 100.

and Y_2 well interpreted, the variable data of isoniazid release in 0.1 N HCl at 2 h and isoniazid release in pH 6.8 phosphate buffer at 45 min. Thus, the lower magnitude of error (−3.8 to 2.31 for Y_1 and −2.6 to 2.46 for Y_2) as well as significant values of r^2 (>0.9) in the current study indicate the robustness of the mathematical model and high prognostic ability of RSM.

4. Conclusion

A statistical model has been established to predict the release properties of the isoniazid from the site-specific pellets, by simultaneously studying the effect of various hydrodynamic factors in USP dissolution test apparatus 3, using RSM. The 3^2 FFD strategy was found to point out the significant factors affecting drug release from the site-specific release isoniazid pellets, in the considered experimental domain. A set of optimum conditions for dissolution test, with respect to the release of the isoniazid, were found to be 225 ml of dissolution medium with 25 dpm reciprocation rate. High degree of prognosis obtained for 3^2 full factorial design corroborates that RSM is an efficient tool in optimization experiments.

This approach could be applied for other dissolution procedures as well as for other solid dosage forms. Examination of dissolution data discussed in this work will help research scientist in collection of scientifically sound data and its interpretation.

Acknowledgements

The authors are thankful to The Nagai Foundation (Japan) for providing financial aid for carrying out the research work. The generosity of M/s Cadila Pharmaceuticals Pvt. Ltd. (Ahmedabad, India) and Colorcon Asia Pvt. Ltd. (Mumbai, India) is gratefully acknowledged for providing the gift samples of isoniazid and Acryl-EZE®, respectively.

References

[1] WHO, Treatment of Tuberculosis. Guidelines for National Programmes, WHO, WHO/TB/97, World Health Organization, Geneva (1997) 220.
[2] G.A. Brewer, Isoniazid, in: K. Florey (Ed.), Analytical Profiles of Drug Substances and Excipients, vol. 6, Academic Press, London, 1977, pp. 183–258.
[3] C.J. Shishoo, S.A. Shah, I.S. Rathod, S.S. Savale, Impaired bioavailability of rifampicin from fixed dose combination formulations with isoniazid, Indian J. Pharm. Sci. 63 (2001) 443–449.
[4] S. Singh, T.T. Mariappan, Regional gastrointestinal permeability of rifampicin and isoniazid (alone and their combination) in the rat, Int. J. Tuberc. Lung Dis. 7 (2003) 797–803.
[5] J. Kramer, H. Blume, Biopharmaceutical aspects of multiparticulates, in: I.G. Sellassie (Ed.), Multiparticulate Oral Drug Delivery, vol. 65, Marcel Dekker, Inc., New York, 1994, pp. 307–355.
[6] C.D. Melia, N. Washington, C.G. Wilson, Advantages and disadvantages of multiparticulate delivery systems, in: C.D. Melia, N. Washington, C.G. Wilson (Eds.), Multiparticulate Oral Dosage Forms: Technology and Biopharmaceutics, Scottish academic press, Edinburgh, 1994, pp. 135–140.
[7] C.J. Shishoo, S.S. Savale, S.A. Shah, I.S. Rathod, P.K. Mukherjee, In vitro–in vivo correlation of different modified release formulations of theophylline, Indian J. Pharm. Sci. 64 (2002) 222–232.
[8] B. Hoener, L.Z. Benet, Factors affecting drug absorption and drug availability, in: G.S. Banker, C.T. Rhodes (Eds.), Modern Pharmaceutics, vol. 121, Marcel Dekker Inc., New York, 2005, pp. 93–118.
[9] I. Borst, S. Ugwu, A.H. Beckett, New and extended application for USP drug release apparatus 3, Dissolut. Technol. (1997) 1–6.
[10] D. Wong, S. Larrabee, K. Clifford, J. Tremblay, D.R. Friend, USP Dissolution apparatus 3 (reciprocating cylinder) for screening of guar-based colonic delivery formulations, J. Control. Release 47 (1997) 173–179.
[11] J. Li, L. Yang, S.M. Ferguson, T.J. Hudson, S. Watanabe, M. Katsuma, J.A. Fix, In vitro evaluation of dissolution behavior for a colon-specific drug delivery system (CODES™) in multi-pH media using United States pharmacopoeia apparatus 2 and 3, AAPS Pharm. Sci. Technol. 3 (2002) article 33. (http://www.aapspharmscitech.org).
[12] J. Cacace, E.E. Reilly, A. Amann, Comparison of the dissolution of metaxalone tablets (Skelaxin) using USP apparatus 2 and 3, AAPS Pharm. Sci. Technol. 5 (2004), article 6. (http://www.aapspharmscitech.org).
[13] S. Missaghi, R. Fassihi, Release characterization of dimenhydrinate from an eroding and swelling matrix: selection of appropriate dissolution apparatus, Int. J. Pharm. 293 (2005) 35–42.
[14] S. Furlanetto, F. Maestrelli, S. Orlandini, S. Pinzauti, P. Mura, Optimization of dissolution test precision for a ketoprofen oral extended-release product, J. Pharm. Biomed. Anal. 32 (2003) 159–165.
[15] B. Singh, S.K. Chakkal, N. Ahuja, Formulation and optimization of controlled release mucoadhesive tablets of atenolol using response surface methodology, AAPS Pharm. Sci. Technol. 7 (2006), article 3. (http://www.aapspharmscitech.org).
[16] M. Kincl, S. Turk, F. Vrečer, Application of experimental design methodology in development and optimization of drug release method, Int. J. Pharm. 291 (2005) 39–49.
[17] A. Kramar, S. Turk, F. Vrečer, Statistical optimization of diclofenac sustained release pellets coated with polymethacrylic films, Int. J. Pharm. 256 (2003) 43–52.
[18] US Pharmacopoeia 30/NF25. US Pharmacopoeial Convention, Rockville, MD (2007) 1691.
[19] US Pharmacopoeia 30/NF25. US Pharmacopoeial Convention, Rockville, MD (2007) 2413.
[20] R. Rastogi, Y. Sultana, M. Aqil, A. Ali, S. Kumar, K. Chuttani, A.K. Mishra, Alginate microspheres of isoniazid for oral sustained drug delivery, Int. J. Pharm. 334 (2007) 71–77.
[21] K. Wingstrand, B. Abrahamsson, B. Edgar, Bioavailability from felodipine extended-release tablets with different dissolution properties, Int. J. Pharm. 60 (1990) 151–156.
[22] P.O. Quist, G.Ö. Stling, Accelerated dissolution testing for improved quality assurance, J. Pharm. Biomed. Anal. 28 (2002) 1081–1089.
[23] C. Becker, J.B. Dressman, G.L. Amidon, H.E. Junginger, S. Kopp, K.K. Midha, V.P. Shah, S. Stavchansky, D.M. Barends, Biowaiver monographs for immediate release solid oral dosage forms: isoniazid, J. Pharm. Sci. 96 (2007) 522–531.

International Journal of Pharmaceutics 388 (2010) 64–72

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Multivariate optimization of formulation and process variables influencing physico-mechanical characteristics of site-specific release isoniazid pellets

Swati Pund, Amita Joshi, Kamala Vasu, Manish Nivsarkar, Chamanlal Shishoo*

B.V. Patel PERD Centre, Department of Pharmaceutics, Sarkhej-Gandhinagar Highway, Thaltej, Ahmedabad 380054, Gujarat, India

ARTICLE INFO

Article history:
Received 24 April 2009
Received in revised form 9 December 2009
Accepted 16 December 2009
Available online 24 December 2009

Keywords:
Isoniazid
Multiple response optimization
Pellets
Physico-mechanical characteristics
Full factorial design

ABSTRACT

In the present study, isoniazid was formulated as site-specific release pellets with high drug loading (65%, w/w) using extrusion-spheronization followed by aqueous coating of Sureteric® (35% weight gain). A statistical experimental strategy was developed to optimize simultaneously the effect of the two formulation variables and one process variable on the critical physico-mechanical properties of the core pellets of isoniazid. Amount of granulating fluid and amount of binder were selected as formulation variables and spheronization speed as a process variable. A 2^3 full factorial experimental design was employed for the present study. Pellets were characterized for physico-mechanical properties viz. usable yield, pellet size, pellips, porosity, abrasion resistance, mechanical crushing force, residual moisture and dissolution efficiency. Graphical and mathematical analysis of the results allowed the identification and quantification of the formulation and process variables active on the selected responses. A polynomial equation fitted to the data was used to predict the responses in the optimal region. The optimum formulation and process parameters were found to be 44.24% (w/w) of granulating fluid, 2.13% (w/w) of binder and spheronization speed of 1000 rpm. Optimized formulation showed usable yield 84.95%, particle size 1021.32 μm, pellips 0.945, porosity 46.11%, and abrasion resistance 0.485%. However, mechanical crushing force, residual moisture and dissolution efficiency were not significantly affected by the selected independent variables. These results demonstrate the importance of, amount of water, binder and spheronization speed, on physico-mechanical characteristics of the isoniazid core pellets with high drug loading.

1. Introduction

Isoniazid, in combination with rifampicin, is used as a first-line drug for the treatment of tuberculosis. However, poor and impaired bioavailability of rifampicin from a number of dosage forms of rifampicin and its combination with isoniazid continues to be a subject of much concern (Shishoo et al., 2001a,b). Earlier studies have established that in the acidic pH of stomach rifampicin reacts with isoniazid to form an inactive compound, isonicotinyl hydrazone resulting in reduction of bioavailability of rifampicin to the extent of 30%. Further, the solid–solid interaction between the two drugs in a fixed dose combinations degrades rifampicin to the extent of 10%. Hence, there is an urgent need to develop an oral system, which will directly address the issues of unacceptable rifampicin bioavailability. The fabrication of a multiparticulate formulation of principal anti-TB drugs which attains segregated delivery of rifampicin and isoniazid for improved rifampicin bioavailability could be a step in the right direction (Shishoo et al., 2001b; Singh et al., 2001; Toit et al., 2006). Since, isoniazid occurs in the protonated form at acidic pH ($pK_a = 2$), it is less permeated through the stomach and is mainly absorbed through the intestine (Mariappan and Singh, 2003). Therefore, isoniazid was formulated for site-specific release in intestine.

Considering the popularity and the robustness of the multiparticulate system (e.g., pellets, granules, etc.) as a means of tailoring the release profile of a drug, this approach has been adopted, in the formulation of isoniazid pellets. Pellets offer various advantages over single unit dosage form including minimal risk of dose dumping, flexibility of blending units with different release patterns, as well as short and reproducible gastric residence time (Kramer and Blume, 1994; Melia et al., 1994).

Extrusion-spheronization process is the most widely accepted method of pellet manufacturing (Ghebre-Sellassie and Knoch, 2007). However, it is likely to fail when slight changes in formulation and process are made. Nevertheless, pelletization is a rather complicated multivariable process (Hellén et al., 1993; Sousa et al., 1996; Neau et al., 2000). A large number of factors, including the physico-chemical properties of the raw materials, both drug and excipients, the composition and the component's relative amounts in the formulations, as well as the manufacturing process parameters, can influence various properties of the formulation (Sousa et al., 1996, 2002). Thus, identifying the influence of these vari-

* Corresponding author. Tel.: +91 79 27439375; fax: +91 79 27450449.
E-mail address: perd@perdcentre.com (C. Shishoo).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.12.034

ables is especially essential in achieving a controlled process and the product.

Multivariate optimization methodologies are powerful, efficient and systematic tools in the design of pharmaceutical dosage forms, allowing a rational study of the influence of formulation and/or processing parameters on the selected responses with a shortening of the experimentation time and an improvement in the quality of research and development work (Furlanetto et al., 2003, 2006; Kramar et al., 2003; Singh et al., 2006; Kim et al., 2007; Joshi et al., 2008). Experimental design is thus the preferred strategy, especially when complex formulations, such as multiparticulate systems, are to be developed (Neau et al., 2000). Multivariate optimization methodologies has been successfully applied in developing multiple-unit delivery systems, allowing a rapid and efficient quantification and prediction of the effects of formulation changes on the considered responses (Neau et al., 2000; Gupta et al., 2001; Paterakis et al., 2002; Akhgari et al., 2005; Howard et al., 2006).

Considerable amount of work has been reported which identifies the factors involved in pelletization, still there are areas of uncertainty remaining; especially for pellets with high drug loading. The ability to produce pellets with high drug loading is one of the claimed advantages of the process of extrusion-spheronization, but it is not possible to achieve this with all the drugs, especially those with very high aqueous solubility. There are very few reports which provide evidence for the ability to prepare pellets with high drug loading using Avicel PH 101, although various other grades such as Avicel RC 591, Avicel 955 have been suggested as alternatives for successful pelletization (Jover et al., 1996; Podczeck et al., 2008).

The present study deals with the core pellet optimization with high drug loading for isoniazid The objective of this work was to establish the effect of formulation as well as process variables and their eventual interactions over various micromeritic, mechanical and release characteristics of the isoniazid pellets. In order to do so, the experimental plan chosen was the 2^3 full factorial analysis along with graphical interpretation of the effects and mathematical modelling. Two formulation variables; amount of granulating fluid and the binder concentration and a process variable; spheronization speed were studied.

2. Materials and methods

2.1. Materials

Isoniazid was received as gift sample from Litaka Pharmaceuticals Pvt. Ltd., Pune, India. Microcrystalline cellulose (Avicel® PH 101, Signet Chemical Corporation, Mumbai, India), polyvinylpyrrolidone (Kollidon® 90, BASF, Germany) and Sureteric® (Colorcon Asia Pvt. Ltd. Mumbai, India), were used as excipients and obtained from the indicated sources. All other ingredients and reagents were of analytical grade and were used as received.

2.2. Preparation of isoniazid core pellets

2.2.1. Experimental design

Before application of the design, a number of preliminary trials were conducted to determine the conditions at which the process resulted to pellets. The levels of the factors were also determined by this procedure.

A 2^3 full factorial design was used for optimizing the formulation. The studied factors were: the amount of granulating fluid; purified water (X_1, % w/w of dry blend) and amount of binder; Kollidon® 90 (X_2, % w/w of dry blend) and the spheronization speed (X_3, revolutions per minute, rpm). The responses studied were usable yield (Y_1, %theoretical), pellet size (Y_2, μm), pellips (Y_3), porosity (Y_4, %), abrasion resistance (Y_5, %), mechanical crushing force (Y_6, N), residual moisture (Y_7, %) and dissolution efficiency (DE) at 15 min (Y_8, %). These studied factors along with their levels and the corresponding responses are summarized in Table 1 and experimental formulations are listed in Table 2.

2.2.2. Preparation of isoniazid core pellets

Powder components of the formulation (Isoniazid, 65% (w/w); Avicel® PH 101, 32–35% (w/w) and Kollidon® 90,0–3% (w/w)) were mixed in a small-scale planetary mixer (Kalweka, Karnavati Eng. Ltd., India) for 10 min. The required quantity of water (35–55%, w/w of dry powder blend) was added as per the factorial design. The wet mass was processed for further 10 min with occasional pauses to allow scraping of the bowl and blade. Extrudates were obtained by using gravity fed cylinder extruder (R.R. enterprises, India), extruding at a constant speed of 125 rpm, through a roller die having holes 1 mm in diameter and 4 mm in length. A spheronizer (R.R. enterprises, India), equipped with a rotating plate of regular crosshatch geometry was used for the spheronization. The extrudates were spheronized for 10 min at speed (700–1000 rpm) as per the experimental design. The contents emptied from the spheronizer were dried in the Fluidized Bed dryer (Niro-Aeromatic, Switzerland) at 50 °C for 20 min.

2.2.3. Characterization of uncoated isoniazid pellets

2.2.3.1. Usable yield (% theoretical). The size distribution of uncoated pellets was determined by sieving using standard set of sieves (600–2360 μm) on a sieve shaker (Electromagnetic sieve shaker, EMS-8, Electrolab, India) for 5 min at a frequency of 50 Hz with amplitude of 1 mm. The fraction of pellets, 700–1190 μm, was considered as the usable yield (Howard et al., 2006).

2.2.3.2. Pellet size. Particle size for each batch was determined using Laser Light Scattering system (Malvern Mastersizer 2000, Malvern Instruments, Malvern, UK). All the measurements were carried out in triplicate and 50th percentile diameter of the cumulative particle size distribution was considered as mean pellet size (Koo and Heng, 2001).

2.2.3.3. Determination of the shape using image analysis. For shape analysis, the images were captured using a stereomicroscope Leica S4E (Leica, Germany). The captured images were analyzed using Image analysis software (AnalySIS®, Soft Imaging system, v. 5.2, Münster, Germany). Analysis was carried out on 50 pellets from usable yield fraction. In this study, pellips was calculated for the characterization of the shape by using the following equation (Koo and Heng, 2001; Almeida-Prieto et al., 2007)

$$\text{pellips} = \frac{P}{\pi \times d_{\max}} \quad (1)$$

where P is the perimeter and $d_{\max}$ is maximum diameter of the pellet, calculated directly by using Image analysis software.

2.2.3.4. Mechanical crushing force. At least 20 pellets from the usable yield fraction of each formulation were evaluated for their diametral crushing force using a tablet strength tester (EH 01, Electrolab, India) (Sousa et al., 2002; Newton et al., 2007).

2.2.3.5. Abrasion resistance. The resistance to abrasion was analyzed using Roche friabilator (Veego instruments corporation, India). A pre-weighed sample (approximately 6 g) taken from the usable yield fraction was placed in a friabilator along with 25 steel spheres, each 2 mm in diameter. After 100 revolutions at 25 rpm, the mass retained on the sieve (1190 μm) was weighed and the abrasion resistance was calculated as the percentage loss of mass

Table 1
2^3 Full factorial experimental design: factors and responses.

Factors	Levels of the factors used in the formulation	
	−1	+1
X_1 = amount of granulating fluid, water	35% (w/w) of dry mix	55% (w/w) of dry mix
X_2 = amount of binder, Kollidon® 90	0% (w/w) of dry mix	3% (w/w) of dry mix
X_3 = Spheronization speed	700 rpm	1000 rpm
Responses		
Y_1 = usable yield (% theoretical)	Y_5 = abrasion resistance (%)	
Y_2 = pellet size (μm)	Y_6 = mechanical crushing force (N)	
Y_3 = pellips	Y_7 = residual moisture (%)	
Y_4 = porosity (%)	Y_8 = dissolution efficiency; DE (%)	

between initial and final weights of each pellet batch (Howard et al., 2006). Each batch was analyzed in triplicate.

2.2.3.6. Porosity. Pellet porosity was determined using Helium pycnometry (SmartPycno 30, Smart Instruments, India). All the values are mean of three replicates (Chopra et al., 2001; Steckel and Mindermann-Nogly, 2004).

2.2.3.7. Residual moisture. The residual water content present in the pellets after drying was determined by USP Method A using Karl Fischer titrator (Systronics Universal titrator 353, India). The equipment was pre-calibrated and standardised with disodium tartrate dihydrate. Pellets, approximately 250 mg, were accurately weighed and immediately placed in the moisture analyser for titration with Karl Fischer reagent. Each batch was analyzed in triplicate (USP 30/NF25, 2007a).

2.2.3.8. Dissolution efficiency; DE. Dissolution study on uncoated pellets was carried out in pH 6.8 phosphate buffer in USP dissolution apparatus I (Hanson Research Corporation, Chatsworth, CA) and DE at 15 min was calculated. Isoniazid released in the dissolution media was measured at λ_{max} 263 nm by a validated spectrophotometric method (Joshi et al., 2008; Rastogi et al., 2007). For each dissolution run, a mean of six determinations was recorded.

2.3. Statistical analysis of the data and validation of the optimization model

The NEMRODW software (LPRAI SARL, Marseille, France) was used in the current study for the generation and evaluation of statistical experimental design. Polynomial models including interaction terms were generated for all the response variables using multiple linear regression analysis. The influence of factors and their interaction, on each of the response are represented graphically.

In order to validate the polynomial equations, one optimum checkpoint (formulation composition and process) and two random checkpoints were selected by intensive grid search, performed over the entire experimental domain. The criterion for selection of optimum check point was mainly based on the highest possible values of response parameters, i.e. usable yield, porosity, mechanical crushing force, DE and pellips; while lowest possible values of responses, namely, size, abrasion resistance and water content. Formulations corresponding to these three check points were prepared and evaluated for all the eight responses (Y_1–Y_8). The resultant experimental data of response properties were subsequently compared quantitatively with the predicted values.

2.4. Enteric coating of optimized isoniazid core pellets for site-specific release and evaluation of coated pellets

A coating of drug-loaded pellets with optimum composition was carried out with 10% (w/w) aqueous suspension of Sureteric® using fluid-bed coater (Niro-Aeromatic, Switzerland) to achieve 35% weight gain. The process conditions were pre-warming of the cores at 40 °C for 10 min; spray nozzle diameter, 1 mm; atomizing air pressure, 1 bar; air flow rate, 80 $m^3\ h^{-1}$; inlet air temperature, 40 °C; product temperature 32–35 °C; spray rate, 1.5 $ml\ min^{-1}$; post-drying at 40 °C for 10 min.

2.4.1. Dissolution testing of site-specific release isoniazid pellets

The enteric-coated isoniazid pellets were characterized for the complete release profile. Method B for delayed release products, specified in USP, was followed (USP 30/NF 25, 2007b). All the dissolution samples were analyzed immediately after the completion of dissolution test by UV–vis spectrophotometer (Shimadzu UV-2450, UV–vis scanning spectrophotometer, Japan) (Joshi et al., 2008). For each dissolution run, a mean of six determinations was recorded.

2.4.2. Surface topography

Morphological examination of the surface of uncoated as well as coated pellets of optimized isoniazid formulation was carried out using a scanning electron microscope. Scanning electron microphotographs of pellets were obtained using JEOL (JEOL JSM-6100, Tokyo, Japan). The particles were vacuum dried, coated with thin gold–palladium layer by sputter coater unit (JEOL JFM-1100, Tokyo, Japan) and observed microscopically at an accelerating voltage of 5.0 kV.

Table 2
Formulations as per the 2^3 Full Factorial experimental design.

Formulation run	Formulation variable - X_1 (amount of granulating fluid, % w/w of dry mix)	Formulation variable - X_2 (amount of binder, % w/w of dry mix)	Process variable - X_3 (spheronization speed, rpm)
1	−1	−1	−1
2	+1	−1	−1
3	−1	+1	−1
4	+1	+1	−1
5	−1	−1	+1
6	+1	−1	+1
7	−1	+1	+1
8	+1	+1	+1

3. Results and discussion

3.1. Preliminary experiments

The purpose of pelletization process is to produce spherical particles of acceptable size and size distribution along with good mechanical strength and desired release properties. The common way for the delivery of pellets is by filling them in hard gelatin capsules. Also, they may be coated to produce desired drug release profile. Therefore, it is important to determine the pellet size, size distribution, shape, abrasion resistance and mechanical strength as these parameters determine the quality of pellets produced. Also, filling in hard gelatin capsules is uniform, and their coating procedure becomes successful (Paterakis et al., 2002). Although, with Avicel PH 101 formulations, there did not appear to be an issue with the ability to make pellets, our preliminary work revealed that the amount of granulating fluid and the binder along with spheronization speed are significant variables influencing the formulation of isoniazid pellets. This may be attributed to high drug loading and low level of Avicel PH 101. Thus, optimization of core isoniazid pellets was further investigated in depth in order to draw maximum advantage from its potential effectiveness for site-specific release.

In the present study, experimental design methodology was exploited systematically for evaluating the effect of varying the amount of granulating fluid; water, binder; Kollidon® 90, and spheronization speed as well as to highlight any interaction among the components on the micromeritic, mechanical and release properties of isoniazid pellets. This will facilitate the identification of the most significant factors influencing these properties and establishing their best levels for optimizing the considered experimental responses.

Mathematical relationship was generated between the factors and responses for determining the levels of factors, which yield optimum responses. A first order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

where b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of eight experimental runs; b_1–b_3 are the coefficients computed from the observed experimental values of Y; and X_1, X_2 and X_3 are the coded levels of factors. The terms X_iX_j (i and j = 1, 2 and 3) represent the interaction terms. The equation represents the quantitative effect of factors (X_1, X_2 and X_3) upon each of the responses; Y_1–Y_8. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. A positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors.

Analysis of variance (ANOVA) was applied for estimating the significance of the model, at 5% significance level. A model is considered significant if the p-value is less than 0.05. In addition, graphical analysis of responses was carried out. This analysis allowed the important factors for the considered responses to be pointed out and the optimum factor level to be selected. The bar graphs were constructed in which the bars that exceed the two reference lines, calculated according to the experimental variance, correspond to the factors that are active on the response. In particular, the active factors are those where a level change determines a response variation which is statistically different from the variation due to the experimental error (Furlanetto et al., 2003, 2006).

3.2. Usable yield, pellet size and size distribution

For a successful extrusion-spheronization process and the formulation, a high percentage of pellets should be produced within a desired size range. Pellets prepared by the process of extrusion and spheronization generally have a mean size between 0.5 and 1.5 mm depending on the diameter of the hole in the extruder die plate. Size polydispersity of pellets is an important factor to be considered if the pellets are to be coated for modifying the release of the drug. The uniformity of the coating requires narrow and homogeneous pellet size distribution that remains unchanged from batch to batch. The size and size distribution of pellets also affect the coating capture by the cores and thereby the release kinetics of the drugs (Mehta, 1989). In order to decrease the variation in fill weight, Rowe et al. (2005) have emphasized the importance of narrow pellet size distribution using computer simulation studies.

Table 3
Result data of mean values of various responses, i.e. usable yield (% theoretical, Y_1), pellet size (μm, Y_2), pellips (Y_3), porosity (%, Y_4), abrasion resistance (%, Y_5), mechanical crushing force (N, Y_6), residual moisture (%, Y_7) and dissolution efficiency at 15 min (%, Y_8).

Formulation run	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
1	95.5	707.25	0.861	48.81	2.00	5.5	1.889	65.5
2	84.8	1016.06	0.888	46.32	1.50	5.8	1.795	65.8
3	85.2	1003.26	0.873	45.94	0.20	8.2	1.870	68.2
4	80.9	1108.43	0.892	43.71	0.10	8.9	1.876	71.9
5	94.7	750.23	0.933	43.99	1.60	4.7	1.798	70.7
6	87.3	979.12	0.952	42.86	1.30	5.3	1.703	65.3
7	83.5	1063.76	0.933	43.91	0.20	8.5	1.810	68.5
8	81.3	1107.5	0.941	43.45	0.00	8.3	1.780	65.3

Our results indicated that increasing the concentration of binder, increases the mean pellet size but decreases the yield in the desirable size range; 700–1190 μm (Table 3). Usable yield appears to be inversely influenced by the amount of binder as well as amount of granulating fluid (p-value <0.05, Table 4); however, the response is more dominated by the amount of binder as seen in Eq. (3) and Fig. 1a. This may be because of higher cohesiveness and inter-particulate adherence provided to the wet mass by binder and granulating fluid, resulting in pellet agglomeration. This agglomeration increases the quantity of abnormally large particles thereby, decreasing the usable yield.

The largest pellets are the ones made with high amount of binder along with higher amount of granulating fluid. This clearly implies that the solvent influences the packing of the particles during processing. This is evident from the highest positive value of coefficient of term X_2 (Eq. (4)) and highest bar length seen in graphical analysis (Fig. 1b). In addition to X_2, X_1 and the interaction term X_1X_2, significantly contribute to the pellet size as evident from the p-values of their coefficients (Table 4). However, negative influence of X_1X_2 can be attributed to the potential occurrence of interaction between the factors, construing that each factor is tending to modify the effect of another towards the pellet size. Nevertheless, all the batches demonstrated narrow size distribution and followed Gaussian curve

$$Y_1 = 86.65 - 3.075X_1 - 3.925X_2 + 0.050X_3 + 1.45X_1X_2 + 0.675X_1X_3 - 0.375X_2X_3 \quad (3)$$

where F = 204.17, p = 0.0435 and r^2 = 0.994

$$Y_2 = 966.951 + 85.826X_1 + 103.786X_2 + 8.201X_3 - 48.599X_1X_2 - 17.669X_1X_3 + 6.691X_2X_3 \quad (4)$$

where F = 652.82, p = 0.0300 and r^2 = 0.998.

3.3. Shape analysis

If the process of extrusion and spheronization is not optimized, pellet shapes can vary ranging from rounded cylinders to dumbbells and ellipsoids. It is desirable to obtain high usable yield of durable pellets, it is ultimately the shape of the collected mate-

Table 4
A summary of *p*-values for coefficients of factors for response: Y_1 (usable yield), Y_2 (pellet size), Y_3 (pellips), Y_4 (porosity), Y_5 (abrasion resistance), Y_6 (mechanical crushing force), Y_7 (residual moisture) and Y_8 (dissolution efficiency at 15 min).

Coefficient	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
b_1	**0.0310**	**0.0171**	**0.0261**	**0.0416**	0.1695	0.4511	0.1037	0.1625
b_2	**0.0243**	**0.0142**	0.3440	0.0530	**0.0323**	0.0604	0.1450	0.1145
b_3	0.7952	0.1749	**0.0078**	**0.0249**	0.2578	0.4097	0.0655	0.4097
b_{12}	0.0656	**0.0303**	0.1000	0.2650	0.3440	0.7952	0.1331	0.1344
b_{13}	0.1392	0.0828	0.1000	0.0830	0.7952	0.7048	0.4823	0.0604
b_{23}	0.2422	0.2117	0.0700	**0.0438**	0.3440	0.5577	0.5817	0.0692

Significant effects of factors ($p < 0.05$) on individual responses are shown in bold type.

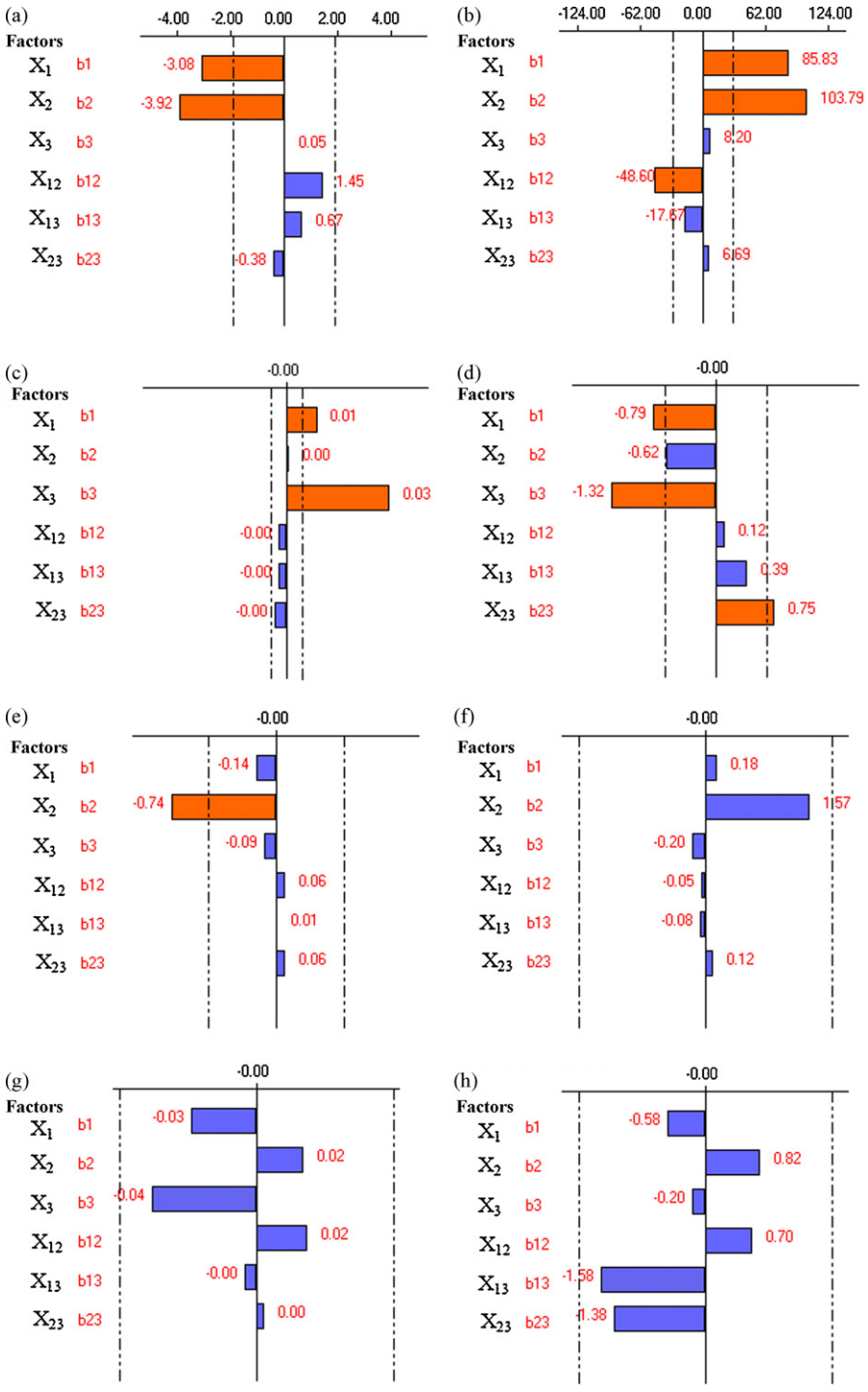


Fig. 1. Graphical representation of effect of factors on various responses (Y). (a) Usable yield (Y_1); (b) pellet size (Y_2); (c) pellips (Y_3); (d) porosity (Y_4); (e) abrasion resistance (Y_5); (f) mechanical crushing force (Y_6); (g) residual moisture (Y_7) and (h) DE (Y_8).

rial that is critical for a number of processing advantages (e.g., a free flowing and uniformly coated product). It is eventually the work of spheronization process, to fragment the extrudate through interactions with the frictional plate, and subsequently smoothen the fragments into the spherical pellets. A variety of parameters have been used to express the shape of the pellets like aspect ratio, roundness score, circularity, pellips, elongation, projection sphericity, etc. (Podczeck et al., 1999; Steckel and Mindermann-Nogly, 2004; Howard et al., 2006; Almeida-Prieto et al., 2007). For the current study, pellips was used for the characterization of shape of pellets. A pellips of 1 represents a perfect sphere. In the present study, pellips ranged from 0.861 to 0.952 (Table 3). The pellets produced with low amount of granulating fluid and low spheronization speed were comparatively non-spherical.

The regression equation for the pellips is

$$Y_3 = 0.909 + 0.009X_1 + 0.0006X_2 + 0.031X_3 - 0.002X_1X_2 - 0.002X_1X_3 - 0.003X_2X_3 \quad (5)$$

where $F = 1237.59$, $p = 0.0218$ and $r^2 = 0.999$.

Our graphical analysis (Fig. 1c) shows that pellips is significantly affected by the amount of granulating fluid; water and the spheronization speed (p-value < 0.05, Table 4). This is ascribed to the fact that rounding of pellets in the spheronizer is a function of plasticity of the extrudates, where water acts as a plasticizer (Lustig-Gustafsson et al., 1999). During spheronization agglomerates undergo densification resulting in increased availability of surface water and in increased surface plasticity. This will allow faster rounding of extrudates but excessive surface water will result in further pellet growth (Heng and Koo, 2001).

3.4. Pellet porosity

Pellet porosity, a vital characteristic, strongly depends on composition of pellet, volume of the wetting liquid, spheronization and drying conditions. This will critically determine the relevant properties such as friability, flowability, wettability, adhesion to various substrates and drug release profile in different ways. This also has the potential to change the ability of a film to adhere to the surface of the pellets (Gómez-Carracedo et al., 2009). Values of porosity for all the eight batches range from 42.86% to 48.81% (Table 3)

$$Y_4 = 44.874 - 0.7894X_1 - 0.621X_2 - 1.321X_3 + 0.116X_1X_2 + 0.391X_1X_3 + 0.749X_2X_3 \quad (6)$$

where $F = 217.61$, $p = 0.0251$ and $r^2 = 0.995$.

There appears to be significant negative influence of individual components, i.e. amount of granulating fluid and spheronization speed on the porosity of the isoniazid pellets (p-value <0.05, Table 4); as depicted in the graph (Fig. 1d and Eq. (6)). This can be explained by the fact that during spheronization of extrudates, water migrates to the surface resulting in reduction of voids, which in turn, leads to further densification and reduced porosity.

3.5. Abrasion resistance and mechanical crushing force

Abrasion resistance is designed to assess the resistance of the pellet surface to abrasion, which pellets will encounter during further processing and shipping, whereas, mechanical crushing force gives indication of its mechanical robustness. The values for both the parameters are shown in Table 3. Pellets with high resistance to abrasion are desirable, as they are likely to retain their integrity on handling and during further processing, such as coating. The regression equations for abrasion resistance and mechanical crushing force are shown as Eqs. (7) and (8), respectively

$$Y_5 = 0.862 - 0.137X_1 - 0.737X_2 - 0.088X_3 + 0.062X_1X_2 + 0.013X_1X_3 - 0.062X_2X_3 \quad (7)$$

where $F = 68.56$, $p = 0.0092$ and $r^2 = 0.983$.

The amount of binder was found to have significant influence on abrasion resistance ($p < 0.05$) as shown in Table 4. Graphical analysis (Fig. 1e) and Eq. (7) reveals that amount of binder is inversly affecting the abrasion resistance. This implies that in order to minimize the abrasion resistance, amount of binder needs to be maximized. Pellets lacking sufficient binding property at the surface will experience greater damage during attrition and make them more vulnerable to wear and tear

$$Y_6 = 6.9 + 0.175X_1 + 1.575X_2 - 0.200X_3 - 0.050X_1X_2 - 0.075X_1X_3 + 0.125X_2X_3 \quad (8)$$

where $F = 19.07$, $p = 0.0173$ and $r^2 = 0.939$.

As far as, mechanical strength of individual pellet is concerned, values for all the eight batches are comparable and contribution of each factor and their interaction was found to be statistically non-significant ($p > 0.05$, Table 4). This might be due to the fact that strength measurements were carried out on pellets of same size fraction, 0.8–1.0 mm.

3.6. Residual moisture

Uncoated pellets of all the eight runs were found to have had lower moisture content (1.7–1.9%; Table 3). This is in agreement with the graphical representation (Fig. 1g), in which none of the factor or their cross-product term were found to be significant (Eq.

Table 5
The experimental and predicted values for all the eight responses (Y_1–Y_8) along with percentage prediction error[a] observed for optimum formulation (A) and random formulation (B and C).

Response	A (44.24, 2.13, 1000)[b]			B (46.76, 1.7, 1000)[b]			C (42.55, 1.59, 780)[b]		
	Experimental value	Predicted value	% Prediction error[a]	Experimental value	Predicted value	% Prediction error[a]	Experimental value	Predicted value	% Prediction error[a]
Y_1	84.95	85.01	−0.07	84.74	85.74	−1.18	87.68	87.21	0.54
Y_2	1021.32	1018.29	0.29	996.11	1000.66	−0.46	955.101	946.835	0.87
Y_3	0.945	0.938	0.74	0.944	0.940	0.42	0.889	0.892	−0.34
Y_4	46.11	45.02	2.36	44.21	45	−1.79	44.00	45.04	−2.36
Y_5	0.485	0.496	−2.26	0.671	0.66	1.64	0.922	0.891	3.36
Y_6	7.32	7.413	−1.27	7.01	6.94	0.99	6.91	7.03	−1.74
Y_7	1.729	1.783	−3.12	1.73	1.77	−2.31	1.86	1.841	1.02
Y_8	68.25	67.35	1.31	67.35	67.01	0.51	67.22	67.78	−0.83

[a] Percent prediction error was calculated using the formula (experimental value − predicted value)/experimental value × 100.
[b] The values represented in the brackets are the amount of granulating fluid in %w/w, amount of binder in %w/w and the speed of spheronization in rpm, respectively for A, B and C formulations.

(9) and Table 4)

$$Y_7 = 1.815 - 0.027X_1 + 0.019X_2 - 0.042X_3 + 0.021X_1X_2 - 0.005X_1X_3 + 0.003X_2X_3 \quad (9)$$

where $F = 28.89$, $p = 0.014$ and $r^2 = 0.959$.

For the standard drying conditions, it was found that, the differences in the residual moisture of the pellets was very small (statistically non-significant), indicating that in spite of the different initial water contents of the pellets, the drying process efficiently removed the free water added during the initial wet massing stage.

3.7. Dissolution efficiency (DE)

DE is a model-independent parameter widely employed as a significant index of drug dissolution performance (Costa and Lobo, 2001; Menegola et al., 2007). In the present study, all the formulation batches showed statistically non-significant and comparable DE. *p*-Values of each coefficient indicate non-significant effect of the individual factors or their interactions on the response (Table 4). This behaviour can be attributed to the highly soluble nature of isoniazid (~125 mg ml^{-1}), which is a borderline of Class I and Class III of BCS (Becker et al., 2007)

$$Y_8 = 67.65 - 0.575X_1 + 0.825X_2 - 0.200X_3 + 0.700X_1X_2 - 1.575X_1X_3 - 1.3753X_2X_3 \quad (10)$$

where $F = 43.79$, $p = 0.0152$ and $r^2 = 0.973$.

3.8. Validation of multiple response optimization model

In order to assess the reliability of the developed mathematical model, formulations corresponding to optimum composition and two additional random compositions covering the entire range of experimental domain were performed. For each of these formulations, the responses were estimated by the use of generated mathematical models and by the experimental procedures. The formulation parameters of the optimum and the random check points, their experimental and predicted values for all the eight response variables are listed in Table 5. The lower magnitudes of the error in current study indicate the robustness of the model and high prognostic ability of multiple response optimization technique.

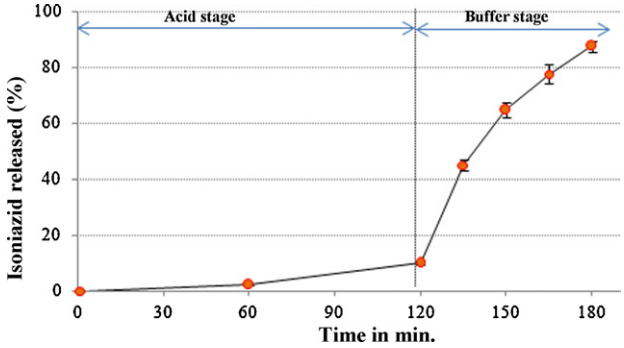


Fig. 2. Complete dissolution profile of site-specific release isoniazid pellets with optimized core formulation and coated with Sureteric® ($n = 6$).

3.9. Analysis of site-specific release coated isoniazid pellets

The isoniazid core pellets formulated using with optimum formulation composition and process condition were evaluated for the above-mentioned physico-chemical properties. The optimum formulation batch composition and the values for its responses results are enlisted in Table 4. The usable yield of core pellets based on the sieve analysis was found to be 84.95%, where as the abrasion resistance and mechanical crushing force were found to be 0.485% and 7.32 N, respectively. This indicted that the core pellets are quite hard and are able to withstand the mechanical stresses of subsequent coating process. Coated pellets had residual moisture 2.59% which is significantly higher than the core pellets. It is possible that the additional moisture content was in the coat. In general, moisture would plasticize the dry film coat making it softer and more flexible.

The complete release profile of site-specific release isoniazid pellets is shown in Fig. 2. Only 10.0% of the isoniazid was released at 120 min in 0.1 N hydrochloric acid which indicates the significant gastric acid resistance of the coated pellets. While isoniazid released in intestinal pH 6.8 buffer was found to be within the acceptance criteria (>85% of the loaded amount). The external morphology of the core and coated pellets, under scanning electron microscope, is shown in Fig. 3a and b, respectively. The coated pellet was spherical with a smoother surface in comparison to core pellet.

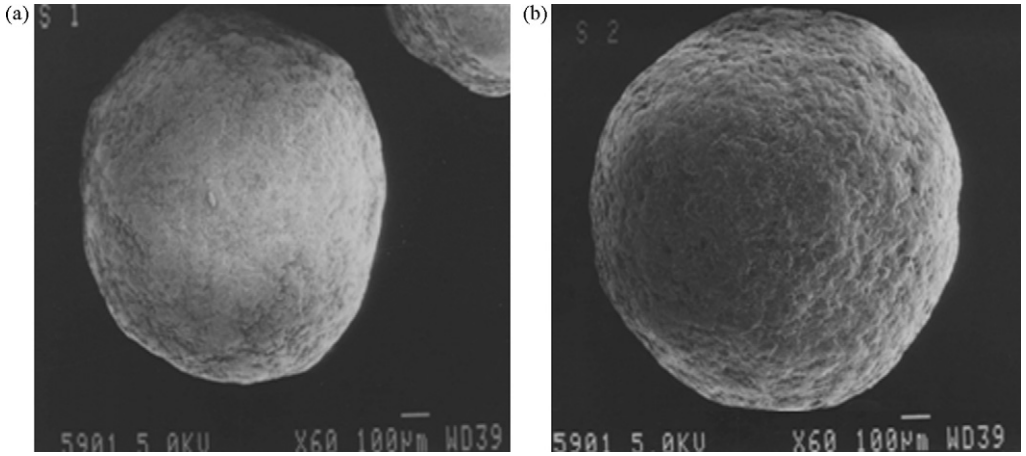


Fig. 3. Scanning electron microphotographs of isoniazid pellets: (a) core pellet; (b) coated pellet

4. Conclusion

In pellets prepared with high drug loading and low level of Avicel PH101, identification of correct level of formulation variables and process variable is essential for desired physico-mechanical properties. Quantitative relationship between the formulation variables, amount of granulating fluid and binder and a process variable, speed of spheronization for the formulation have been identified. In particular, graphical analysis of the effects enabled identification for each examined variable which are active on the selected responses. The mathematical model for each of the response developed using multiple regression analysis quantitatively describes the influence of the selected variables on the responses under study. From the significance of main effects and their interactions found in this work, it was possible to predict the influence of the factors within the defined experimental domain.

A set of optimum parameters for preparing the cores of site-specific release isoniazid pellets with respect to its desired range of physico-mechanical properties were found to be 44.24% (w/w) of granulating fluid, 2.13% w/w of binder and spheronization speed, 1000 rpm. Additional experiments performed at optimal and random variables settings confirmed the validity of the proposed model.

It is evident that, the identification of critical levels of granulating fluid, binder and speed spheronization could be of potential benefit in preparing pellets with high loading of water soluble drug. This approach will help to retain the ability of Avicel PH 101 to prepare satisfactory pellets, even at its low level.

Acknowledgements

The authors are thankful to T. Nagai Foundation (Japan) for providing financial aid to carry out the research work. AJ is thankful to CSIR, New Delhi for the award of Senior Research Fellowship. The generosity of M/s. TwiLight Litaka Pharmaceuticals Pvt. Ltd. (Pune, India) and Colorcon Asia Pvt. Ltd. (Mumbai, India) is gratefully acknowledged for providing the gift samples of isoniazid and Sureteric®, respectively.

References

Akhgari, A., Garekani, H.A., Sadeghi, F., Azimaie, M., 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int. J. Pharm. 305, 22–30.

Almeida-Prieto, S., Blanco-Méndez, J., Otero-Espinar, F.J., 2007. Microscopic image analysis techniques for the morophological characterization of pharmaceutical particles: influence of the software and the factor algorithms used in the shape factor estimation. Eur. J. Pharm. Biopharm. 67, 766–776.

Becker, C., Dressman, J.B., Amidon, G.L., Junginger, H.E., Kopp, S., Midha, K.K., Shah, V.P., Stavchansky, S., Barends, D.M., 2007. Biowaiver monographs for immediate release solid oral dosage forms: isoniazid. J. Pharm. Sci. 96, 522–531.

Chopra, R., Newton, J.M., Alderborn, G., Podczeck, F., 2001. Preparation of pellets of different shape and their characterization. Pharm. Dev. Technol. 6, 495–503.

Costa, P., Lobo, J.M.S., 2001. Modelling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13, 123–133.

Furlanetto, S., Maestrelli, F., Orlandini, S., Mura, P., 2003. Optimization of dissolution test precision for a ketoprofen oral extended-release product. J. Pharm. Biomed. Anal. 32, 159–165.

Furlanetto, S., Cirri, M., Maestrelli, F., Corti, G., Mura, P., 2006. Study of formulation variables influencing the drug release rate from matrix tablet by experimental design. Eur. J. Pharm. Biopharm. 62, 77–84.

Ghebre-Sellassie, I., Knoch, A., 2007. Pelletization techniques. In: Swarbrick, J.E. (Ed.), Encyclopedia of Pharmaceutical Technology, vol. 3. Informa Healthcare, New York, pp. 2651–2663.

Gómez-Carracedo, A., Alvarez-Lorenzo, C., Coca, R., Martínez-Pacheco, R., Concheiro, A., Gómez-Amoza, J.L., 2009. Fractal analysis of SEM images and mercury intrusion porosimetry data for the microstructural characterization of microcrystalline cellulose-based pellets. Acta Mater. 57, 295–303.

Gupta, V.K., Assmus, M.W., Beckert, T.E., Price, J.C., 2001. A novel pH- and time-based multi-unit potential colonic drug delivery system. II. Optimization of multiple response variables. Int. J. Pharm. 213, 93–102.

Hellén, L., Yliruusi, J., Kristoffersson, E., 1993. Process variables of instant granulator and spheronizer: II. Size and size distribution of pellets. Int. J. Pharm. 96, 205–216.

Heng, P.W.S., Koo, O.M.Y.K., 2001. A study of the effects of the physical characteristics of microcrystalline cellulose on performance in extrusion spheronization. Pharm. Res. 18, 480–487.

Howard, M.A., Neau, S.H., Marvin, J.S., 2006. PEO and MPEG in high drug load extruded and spheronized beads that are devoid of MCC. Int. J. Pharm. 307, 66–76.

Joshi, A., Pund, S., Nivsarkar, M., Vasu, K., Shishoo, C., 2008. Dissolution test for site-specific release isoniazid pellets in USP apparatus 3 (reciprocating cylinder): Optimization using response surface methodology. Eur. J. Pharm. Biopharm. 69, 769–775.

Jover, I., Podczeck, F., Newton, M., 1996. Evaluation, by a statistically designed experiment, of an experimental grade of microcrystalline cellulose, Avicel 955, as a technology to aid the production of pellets with high drug loading. J. Pharm. Sci. 85, 700–705.

Kim, M., Kim, J., You, Y., Park, H.J., Lee, S., Park, J., Woo, J., Hwang, S., 2007. Development and optimization of a novel oral controlled delivery system for tamsulosin hydrochloride using response surface methodology. Int. J. Pharm. 341, 97–104.

Koo, O.M.Y.K., Heng, P.W.S., 2001. The influence of microcrystalline cellulose grade on shape and shape distributions of pellets produced by extrusion-spheronization. Chem. Pharm. Bull. 49, 1383–1387.

Kramar, A., Turk, S., Vrečer, F., 2003. Statistical optimization of diclofenac sustained release pellets coated with polymethacrylic films. Int. J. Pharm. 256, 43–52.

Kramer, J., Blume, H., 1994. Biopharmaceutical aspects of multiparticulates. In: Sellassie, I.G. (Ed.), Multiparticulate Oral Drug Delivery, vol. 65. Marcel Dekker Inc., New York, pp. 307–355.

Lustig-Gustafsson, C., Johal, H.K., Podczeck, F., Newton, J.M., 1999. The influence of water content and drug solubility on the formulation of pellets by extrusion and spheronization. Eur. J. Pharm. Sci. 8, 147–152.

Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of rifampicin and isoniazid (alone and their combination) in the rat. Int. J. Tuberc. Lung Dis. 7, 797–803.

Mehta, A.M., 1989. Evaluation and characterization of pellets. In: Ghebre-Sallassie, I. (Ed.), Pharmaceutical Pelletization Technology, vol. 37. Marcel Dekker Inc., New York, pp. 241–265.

Melia, C.D., Washington, N., Wilson, C.G., 1994. Advantages and disadvantages of multiparticulate delivery systems. In: Melia, C.D., Washington, N., Wilson, C.G. (Eds.), Multiparticulate Oral Dosage Forms: Technology and Biopharmaceutics. Scottish Academic Press, Edinburgh, pp. 135–140.

Menegola, J., Steppe, M., Schapoval, E.E.S., 2007. Dissolution test for citalopram in tablets and comparison of in vitro dissolution profiles. Eur. J. Pharm. Biopharm. 67, 524–530.

Neau, S.H., Chow, M.Y., Hileman, G.A., Durrani, M.J., Gheyas, F., Evans, B.A., 2000. Formulation and process considerations for beads containing Carbopol® 974P NF resin made by extrusion-spheronization. Int. J. Pharm. 199, 129–140.

Newton, J.M., Pinto, M.R., Podczeck, F., 2007. The preparation of pellets containing a surfactant or a mixture of mono- and di-glycerides by extrusion/spheronization. Eur. J. Pharm. Sci. 30, 333–342.

Paterakis, P.G., Korakianiti, E.S., Dallas, P.P., Rekkkas, D.M., 2002. Evaluation and simultaneous optimization of some pellets characteristics using 3^3 factorial design and the desirability function. Int. J. Pharm. 248, 51–60.

Podczeck, F., Rahman, S.R., Newton, J.M., 1999. Evaluation of standardised procedure to assess the shape of the pellets using image analysis. Int. J. Pharm. 192, 123–138.

Podczeck, F., Knight, P.E., Newton, J.M., 2008. The evaluation of modified microcrystalline cellulose for the preparation of pellets with high drug loading by extrusion/spheronization. Int. J. Pharm. 350, 145–154.

Rastogi, R., Sultana, Y., Aqil, M., Ali, A., Kumar, S., Chuttani, K., Mishra, A.K., 2007. Alginate microspheres of isoniazid for oral sustained drug delivery. Int. J. Pharm. 334, 71–77.

Rowe, R.C., York, P., Colbourn, E.A., Roskilly, S.J., 2005. The influence of pellet shape, size and distribution on capsule filling—a preliminary evaluation of three-dimensional computer simulation using a Monte-Carlo technique. Int. J. Pharm. 300, 32–37.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Vora, M.J., 2001a. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int. J. Pharm. 228, 53–67.

Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., 2001b. Impaired bioavailability of rifampicin from fixed dose combination formulations with isoniazid. Indian J. Pharm. Sci. 63, 443–449.

Singh, B., Chakkal, S., Ahuja, N., 2006. Formulation and optimization of controlled release mucoadhesive tablets of Atenolol using response surface methodology. AAPS PharmSciTech. 7, E3.

Singh, S., Mariappan, T.T., Sankar, R., Sarda, N., Singh, B., 2001. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from antitubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. Int. J. Pharm. 228, 5–17.

Sousa, J.J., Sousa, A., Podczeck, F., Newton, J.M., Sousa, J.J., 1996. Influence of process conditions on drug release from pellets. Int. J. Pharm. 144, 159–169.
Sousa, A., Podczeck, F., Newton, J.M., 2002. Factors influencing the physical characteristics of pellets obtained by extrusion-spheronization. Int. J. Pharm. 232, 91–106.
Steckel, H., Mindermann-Nogly, F., 2004. Production of chitosan pellets by extrusion/spheronization. Eur. J. Pharm. Biopharm. 57, 107–114.
Toit, L.C., Pillay, V., Danckwerts, M.P., 2006. Tuberculosis chemotherapy: current drug delivery approaches. Respiratory Res. 7, 118.
US Pharmacopoeia 30/NF25, 2007a. US Pharmacopoeial Convention, Rockville, MD, pp. 385.
US Pharmacopoeia 30/NF25, 2007b. US Pharmacopoeial Convention, Rockville, MD, pp. 282.