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 (Fábregas 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 chlorzoxepoxide (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 by Roche during the 1980s (Jansen and Meerwaldt, 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/cm³) 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**  \( \text{C}_{43}\text{H}_{58}\text{N}_4\text{O}_{12} \)
- **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,
Development and evaluation of Rifampicin formulation

- 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 183°C
- 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 β-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.
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 pyrrolidone (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 60°C 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, X₁, %) and amount of soluble extrusion aid (Lactose, X₂, %) were the two factors (independent variables) studied. The responses (dependent variables) studied were porosity (Y₁, %), friability (Y₂, %) and amount of rifampicin released in 45 min. (Y₃, %). 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

<table>
<thead>
<tr>
<th>Factors</th>
<th>Factor level</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>$X_1$ = Amount of Indion (%)</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>$Y_1$ = Porosity (%)</td>
<td>$Y_2$ = Friability (% w/w)</td>
<td></td>
</tr>
<tr>
<td>$X_2$ = Spheronization speed (rpm)</td>
<td>600</td>
<td>800</td>
</tr>
<tr>
<td>$Y_3$ = Amount of rifampicin released in 45 min. (%)</td>
<td></td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Amount of Indion (%)</th>
<th>Spheronization speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>8.00</td>
<td>800</td>
</tr>
<tr>
<td>3</td>
<td>8.00</td>
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<td>800</td>
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<tr>
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<tr>
<td>6</td>
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<td>1000</td>
</tr>
<tr>
<td>8</td>
<td>4.00</td>
<td>600</td>
</tr>
<tr>
<td>9</td>
<td>4.00</td>
<td>800</td>
</tr>
</tbody>
</table>

**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 50th 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 sphericity 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)

\[ \text{Pellips} = \frac{P}{\pi \times d_{\text{max}}} \]  

\[ \text{---(1)} \]

Where, P is the perimeter and \( d_{\text{max}} \) 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)

\[ \% \varepsilon = \left[ \frac{\rho_t - \rho_b}{\rho_b} \right] \times 100 \]  

Where \( \varepsilon \) = effective porosity, \( \rho_t \) = true density and \( \rho_b \) = 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
Formulation development and evaluation of immediate release rifampicin pellets

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
Formulation development and evaluation of immediate release rifampicin pellets

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):

\[
f_2 = 50 \log \left\{ \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^{0.5} \times 100 \right\}
\]

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 dumbbell 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 sphericity 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:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2$$  \hspace{1cm} \text{--(4)}$

\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

<table>
<thead>
<tr>
<th>Source</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.0027</td>
</tr>
<tr>
<td>$X_1$</td>
<td>0.0220</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.0115</td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>0.0476</td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>0.4670</td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>0.0679</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9585</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.8892</td>
</tr>
</tbody>
</table>

*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 spheronation speed can be explained by a mathematical relationship Eq. 5.

\[ Y_1 = 33.60 + 3.61X_1 + 4.58X_2 + 4.63X_1^2 - 1.18X_2^2 + 2.82X_1X_2 \]  

\( \text{---(5)} \)

A positive sign of all the coefficients of the factors i.e., amount of Indion and spheronation speed signifies that the porosity of the pellets increases if the level of these factors increases. The influence of amount of Indion 414 and spheronation 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 spheronation speed affects the porosity in almost a positive and linear fashion. On increasing the amount of Indion 414 and spheronation 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 spheronation. 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 spheronation speed on porosity
**Formulation development and evaluation of immediate release rifampicin pellets**

**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.

\[
Y_2 = 0.46 + 0.043 X_1 + 0.0083 X_2
\]  

---(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

<table>
<thead>
<tr>
<th>Source</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.0426</td>
</tr>
<tr>
<td>X₁</td>
<td>0.0168</td>
</tr>
<tr>
<td>X₂</td>
<td>0.0451</td>
</tr>
<tr>
<td>R²</td>
<td>0.9910</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td>0.9297</td>
</tr>
</tbody>
</table>

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.

\[
Y_3 = 1.04 - 0.010 X_1 + 0.0083 X_2 - 0.017 X_1^2 - 0.11 X_2^2 - 0.00 X_1 X_2
\]  

---(7)

<table>
<thead>
<tr>
<th>Source</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.0112</td>
</tr>
<tr>
<td>X_1</td>
<td>0.3549</td>
</tr>
<tr>
<td>X_2</td>
<td>0.0028</td>
</tr>
<tr>
<td>X_1^2</td>
<td>0.3708</td>
</tr>
<tr>
<td>X_2^2</td>
<td>0.0067</td>
</tr>
<tr>
<td>X_1 X_2</td>
<td>1.0000</td>
</tr>
<tr>
<td>R^2</td>
<td>0.9775</td>
</tr>
<tr>
<td>Adjusted R^2</td>
<td>0.9399</td>
</tr>
</tbody>
</table>

Table 6. ANOVA results for Pellips

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

\[
Y_3 = 71.91 + 8.61 X_1 + 2.54 X_2 - 4.90 X_1^2 + 1.52 X_2^2 + 0.33 X_1 X_2 \quad \text{--(8)}
\]

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.

<table>
<thead>
<tr>
<th>Source</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.0002</td>
</tr>
<tr>
<td>$X_1$</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.0015</td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>0.0011</td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>0.0306</td>
</tr>
<tr>
<td>$X_1 X_2$</td>
<td>0.3216</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9983</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>0.9954</td>
</tr>
</tbody>
</table>

*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
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 (Y₃, pellips=1.00) with maximum porosity (Y₁) and minimum friability (Y₂) along with maximum drug release at 45 min. (Y₄). For, optimization of process, constraints were applied and the optimal calculated parameters were

- Amount of Indion, X₁ = 6.00% w/w
- Spheronization speed, X₂ = 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 (Y₁ to Y₄) along with percentage prediction error* observed for optimum formulation (A) and random formulation (B and C)

<table>
<thead>
<tr>
<th>Formulation (X₁%, X₂ rpm)</th>
<th>Response</th>
<th>Predicted Value</th>
<th>Experimental value</th>
<th>% PE*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Y₁</td>
<td>50.5</td>
<td>50.00</td>
<td>-1.00</td>
</tr>
<tr>
<td><strong>6.00, 800</strong> (Optimum)</td>
<td>Y₂</td>
<td>0.46</td>
<td>0.45</td>
<td>-2.23</td>
</tr>
<tr>
<td></td>
<td>Y₃</td>
<td>0.98</td>
<td>0.99</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Y₄</td>
<td>79.5</td>
<td>81.2</td>
<td>2.09</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Y₁</td>
<td>48.1</td>
<td>46.5</td>
<td>-3.44</td>
</tr>
<tr>
<td><strong>8.00, 10000</strong></td>
<td>Y₂</td>
<td>0.51</td>
<td>0.52</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>Y₃</td>
<td>0.99</td>
<td>0.95</td>
<td>-4.21</td>
</tr>
<tr>
<td></td>
<td>Y₄</td>
<td>80.02</td>
<td>78.5</td>
<td>-1.94</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Y₁</td>
<td>43.5</td>
<td>42.5</td>
<td>-2.35</td>
</tr>
<tr>
<td><strong>4.00, 600</strong></td>
<td>Y₂</td>
<td>0.43</td>
<td>0.40</td>
<td>-7.50</td>
</tr>
<tr>
<td></td>
<td>Y₃</td>
<td>0.88</td>
<td>0.90</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>Y₄</td>
<td>72.1</td>
<td>73.4</td>
<td>1.77</td>
</tr>
</tbody>
</table>

*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

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

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

<table>
<thead>
<tr>
<th>Assay (%)</th>
<th>40°C ± 2°C/75% RH ± 5% RH</th>
<th>At room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDPE</td>
<td>Aluminium strip</td>
</tr>
<tr>
<td>1M</td>
<td>100.45</td>
<td>99.75</td>
</tr>
<tr>
<td>2M</td>
<td>99.53</td>
<td>100.02</td>
</tr>
<tr>
<td>3M</td>
<td>100.67</td>
<td>100.23</td>
</tr>
<tr>
<td>6M</td>
<td>100.43</td>
<td>99.69</td>
</tr>
<tr>
<td>Water Content (%w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>4.11</td>
<td>3.22</td>
</tr>
<tr>
<td>2M</td>
<td>4.47</td>
<td>3.35</td>
</tr>
<tr>
<td>3M</td>
<td>3.65</td>
<td>4.55</td>
</tr>
<tr>
<td>6M</td>
<td>3.88</td>
<td>4.28</td>
</tr>
</tbody>
</table>

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

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 (NaHCO₃), 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 - spheronation
- Rifampicin floating pellets based on effervescent technique
- Rifampicin floating tablet

2.16.1 Approach I- Preparation of rifampicin floating pellets using extrusion-spheronation
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 80m³/h; spray rate 1.5 ml/min.; post drying at 40°C for 10 min. The NaHCO₃-layered pellets were dried in the fluidized bed coater for 30 min. at 50 °C to evaporate the residual moisture.

The NaHCO₃-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, 48-50°C; outlet temperature, 38-40°C; atomizing air pressure, 1 bar; spray rate, 3–5 ml/min. The pellets were further dried in fluidized bed coater at 50°C 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 60°C 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

<table>
<thead>
<tr>
<th>Factors</th>
<th>Factor level</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$=Amount of HPMC K4M</td>
<td>-1, 0, +1</td>
<td>$Y_1$= Floating lag time (min.)</td>
</tr>
<tr>
<td>$X_2$= Amount of Carbopol 971P</td>
<td>5.0, 10.0, 15.0</td>
<td>$Y_2$= Floating duration (h)</td>
</tr>
<tr>
<td></td>
<td>2.0, 6.0, 10.0</td>
<td>$Y_3$= $t_{80%}$</td>
</tr>
</tbody>
</table>
Table 12. Floating rifampicin formulation compositions as per $3^2$ full factorial experimental design

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Amount of HPMC K4M (%)</th>
<th>Amount of Carbopol 971P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.00</td>
<td>10.00</td>
</tr>
<tr>
<td>2</td>
<td>15.00</td>
<td>6.00</td>
</tr>
<tr>
<td>3</td>
<td>15.00</td>
<td>2.00</td>
</tr>
<tr>
<td>4</td>
<td>5.00</td>
<td>6.00</td>
</tr>
<tr>
<td>5</td>
<td>5.00</td>
<td>2.00</td>
</tr>
<tr>
<td>6</td>
<td>15.00</td>
<td>10.00</td>
</tr>
<tr>
<td>7</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>8</td>
<td>10.00</td>
<td>2.00</td>
</tr>
<tr>
<td>9</td>
<td>10.00</td>
<td>6.00</td>
</tr>
</tbody>
</table>

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 37°C ± 0.05° 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 37°C ± 0.05° 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 spectrophotometric 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 (99mTcO₄⁻) 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/m². 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 \((^{99m}\text{TcO}_4^-)\), 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.

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

The rifampicin floating tablets were radiolabelled with 500 microcurie of \(^{99m}\text{TcO}_4^-\) (18.5 MBq). After incorporating \(^{99m}\text{Tc}\) onto rifampicin tablet, \(^{99m}\text{Tc}\) 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 \(^{99m}\text{Tc}\).
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\textsubscript{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):

$$f_2 = 50 \log \left\{ \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i) \right\}^{0.5} \times 100$$

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69
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 \textit{in vitro}. Martínez \textit{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.

\textbf{Table 13}. Evaluation of rifampicin granules of optimized composition

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characteristic of granules</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bulk density (g/cm$^3$)</td>
<td>0.56 ± 0.13</td>
</tr>
<tr>
<td>2</td>
<td>Tapped density (g/cm$^3$)</td>
<td>0.89 ± 0.21</td>
</tr>
<tr>
<td>3.</td>
<td>Hausner ratio</td>
<td>1.75 ± 0.45</td>
</tr>
<tr>
<td>4.</td>
<td>Carr’s Index</td>
<td>10.97± 1.79</td>
</tr>
<tr>
<td>5.</td>
<td>Angle of Repose (°)</td>
<td>28.75±2.01</td>
</tr>
</tbody>
</table>

\textbf{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:

\[ Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2 \]

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 $X_1 X_2$ and $X_i^2$ ($i = 1$ 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.

\[ Y_1 = 3.39 - 0.33 X_1 - 2.50 X_2 - 0.083 X_1^2 + 2.67 X_2^2 + 0.00 X_1 X_2 \]  

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.
Formulation development and evaluation of FDDS of Rifampicin

Table 14. ANOVA results for floating lag time

<table>
<thead>
<tr>
<th>Floating lag time</th>
<th>Source</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model</td>
<td>0.0241</td>
</tr>
<tr>
<td></td>
<td>X₁</td>
<td>0.4022</td>
</tr>
<tr>
<td></td>
<td>X₂</td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td>X₁²</td>
<td>0.2547</td>
</tr>
<tr>
<td></td>
<td>X₂²</td>
<td>0.0205</td>
</tr>
<tr>
<td></td>
<td>X₁ X₂</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.9622</td>
</tr>
<tr>
<td></td>
<td>Adjusted R²</td>
<td>0.8993</td>
</tr>
</tbody>
</table>

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

\[ Y_2 = 6.78 + 1.28 X_1 + 1.33 X_2 -0.62 X_1^2 - 0.97 X_2^2 + 0.12 X_1X_2 \]  

--(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 hydration 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

<table>
<thead>
<tr>
<th>Floating duration</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0.0047</td>
</tr>
<tr>
<td>(X_1)</td>
<td>0.0021</td>
</tr>
<tr>
<td>(X_2)</td>
<td>0.0019</td>
</tr>
<tr>
<td>(X_1^2)</td>
<td>0.0690</td>
</tr>
<tr>
<td>(X_2^2)</td>
<td>0.0223</td>
</tr>
<tr>
<td>(X_1X_2)</td>
<td>0.4838</td>
</tr>
<tr>
<td>(R^2)</td>
<td>0.9875</td>
</tr>
<tr>
<td>Adjusted (R^2)</td>
<td>0.9665</td>
</tr>
</tbody>
</table>

*Significant terms are shown in bold type*
Formulation development and evaluation of FDDS of Rifampicin

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.

\[ Y_3 = 5.61 + 2.12 X_1 + 0.23 X_2 - 0.50 X_1X_2 \] ----(14)

The response surface plot for the influence of amount HPMC K4M and amount of carbopol on t80% 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 t80% 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

<table>
<thead>
<tr>
<th>Source</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>X₁</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>X₂</td>
<td>0.0736</td>
</tr>
<tr>
<td>X₁ X₂</td>
<td>0.0109</td>
</tr>
<tr>
<td>R²</td>
<td>0.9888</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td>0.9820</td>
</tr>
</tbody>
</table>

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 (Y₁ to Y₄) along with percentage prediction error* observed for optimum formulation (A) and random formulation (B and C)

<table>
<thead>
<tr>
<th>Formulation (X₁%, X₂%)</th>
<th>Response</th>
<th>Predicted Value</th>
<th>Expected value</th>
<th>% PE*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Y₁</td>
<td>2.76</td>
<td>2.70</td>
<td>-2.22</td>
</tr>
<tr>
<td>15.44, 7.82 (Optimum)</td>
<td>Y₂</td>
<td>6.14</td>
<td>6.50</td>
<td>5.54</td>
</tr>
<tr>
<td></td>
<td>Y₃</td>
<td>5.89</td>
<td>6.00</td>
<td>1.84</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Y₁</td>
<td>2.99</td>
<td>3.20</td>
<td>6.56</td>
</tr>
<tr>
<td>19.63, 5.17</td>
<td>Y₂</td>
<td>7.71</td>
<td>7.80</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Y₃</td>
<td>7.62</td>
<td>7.50</td>
<td>-1.60</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Y₁</td>
<td>2.40</td>
<td>2.20</td>
<td>-9.09</td>
</tr>
<tr>
<td>10.24, 7.39</td>
<td>Y₂</td>
<td>3.57</td>
<td>3.50</td>
<td>-2.00</td>
</tr>
<tr>
<td></td>
<td>Y₃</td>
<td>3.84</td>
<td>3.90</td>
<td>1.54</td>
</tr>
</tbody>
</table>

*%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. T50 for gastric emptying was calculated graphically, Fig 20. T50 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 T50, 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
**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, 2M, 3M and at 6M 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

<table>
<thead>
<tr>
<th>Assay (%)</th>
<th>40°C ± 2°C/75% RH</th>
<th>At room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDPE</td>
<td>Aluminium strip</td>
</tr>
<tr>
<td>1M</td>
<td>100.35</td>
<td>100.14</td>
</tr>
<tr>
<td>2M</td>
<td>100.07</td>
<td>99.99</td>
</tr>
<tr>
<td>3M</td>
<td>99.67</td>
<td>100.03</td>
</tr>
<tr>
<td>6M</td>
<td>99.43</td>
<td>99.89</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Content (% w/w)</th>
<th>40°C ± 2°C/75% RH</th>
<th>At room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M</td>
<td>4.01</td>
<td>4.25</td>
</tr>
<tr>
<td>2M</td>
<td>4.54</td>
<td>4.15</td>
</tr>
<tr>
<td>3M</td>
<td>4.43</td>
<td>4.22</td>
</tr>
<tr>
<td>6M</td>
<td>4.38</td>
<td>4.18</td>
</tr>
</tbody>
</table>

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

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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


Formulation development and evaluation of FDDS of Rifampicin


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.


