## Literature survey/ Prior art IIIB

### Prior art from Research publications

<table>
<thead>
<tr>
<th>Technique used</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antisolvent precipitation</td>
<td>Ultrafine particles of Rifampicin (RIF) using a RT ionic liquid (1-ethyl 3-methyl imidazolium methyl-phosphonate) as an alternative solvent and a phosphate buffer as an antisolvent (Alessandra et al., 2012)</td>
</tr>
<tr>
<td>Cross linking</td>
<td>Physically crosslinked chitosan microspheres of RIF prepared with sodium tripolyphosphate and sodium hexametaphosphate anion crosslinkers (Gupta and Jabrail, 2007)</td>
</tr>
<tr>
<td>Drug layering</td>
<td>Rifampicin and PVP K-30 dissolved in dichloromethane–methanol mixture and sprayed onto rotating non-pareil beads. The beads were coated with 5% ethyl cellulose (Rao et al., 2001)</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Microemulsion of Rifampicin (RIF) using oleic acid (oil), Tween 80 (surfactant), ethanol (cosurfactant) and pH 7.4 buffer (aqueous phase) (Mehta et al., 2007)</td>
</tr>
<tr>
<td>Emulsification/solvent diffusion</td>
<td>Rifampicin loaded PLGA nanoparticles using polyvinyl alcohol and mixture of dichloromethane and acetone (Esmaeili et al., 2007)</td>
</tr>
<tr>
<td>Extrusion</td>
<td>Immediate release Rifampicin as pellets using MCC, lactose and Polacrilon potassium; extended release Rifampicin as gastroretentive mucoadhesive tablet using Carbopol 71G and HPMC (Pund et al., 2011)</td>
</tr>
<tr>
<td>Spheronisation and dry granulation</td>
<td>PLGA microspheres of Rifampicin using ethyl acetate (Doan et al., 2011)</td>
</tr>
<tr>
<td>Spray drying (four fluid nozzle)</td>
<td>Rifampicin-PLGA nanoparticles containing mannitol microspheres for inhalation therapy (Ohashi et al., 2009)</td>
</tr>
<tr>
<td>Spray drying</td>
<td>PLGA microspheres of Rifampicin (Hirotta et al., 2010)</td>
</tr>
</tbody>
</table>
Two step desolvation | Gelatin nanoparticles using acetone as desolvating agent and gluteraldehyde as a crosslinking agent (Saraogi et al., 2010)
---|---
Wet granulation (non aqueous) | RIF granulated with HPMC (15cps) using isopropyl alcohol (Hiremath and Saha, 2004)

### Prior art from patents

<table>
<thead>
<tr>
<th>Patent</th>
<th>Technique used</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 7,001,893B2 (2006); US 2004/0082541</td>
<td>Complexation with β-cyclodextrin or 2-hydroxypropyl β-cyclodextrin</td>
</tr>
<tr>
<td>US 2013/0115286 A1</td>
<td>Film coating of hard gelatin capsules containing RIF</td>
</tr>
</tbody>
</table>
Rationale IIIB

- Rifampicin (RIF), an antimycobacterial agent for first line therapy of tuberculosis has been classified as a BCS class II drug i.e. poorly soluble and highly permeable.

- Pharmacokinetic considerations
  
i. RIF possesses short biological half life of 3-5 h.
  
ii. Bioavailability of orally administered RIF decreased from 93% after the first single oral dose to 68% after 3 weeks of oral and iv RIF therapy. This is attributed to both, an increased hepatic metabolism and induction of a prehepatic first-pass effect resulted from multiple RIF doses. On first dose administration of 300mg RIF (on empty stomach), the serum concentration curves are similar to those following iv dosing, indicating little presystemic metabolism, but repeated administration induces hepatic endoplasmic reticular enzymes (cytochrome P450s, mainly 3A4 isozyme).

- Immediate release formulations of RIF are released in short duration and are exposed to the acidic environment of the stomach for longer time and thus are highly susceptible to degradation. However, ER formulations of RIF show slower initial release owing to which lesser amount of RIF is available for degradation in acidic medium and hence shows higher concentrations of RIF as the time progresses thereby improving bioavailability (Shishoo et al., 1999; Singh et al., 2001).

- In addition, toxico-allergic side effects and risk of developing microbial resistance are associated with chronic treatment regimen of TB, which can be alleviated by using controlled drug delivery systems.

Objective

The objective was to develop extended release formulations of RIF for once-daily administration to provide a controlled and predictable release of the same using melt extrusion technique.
Experimental IIIB

1. Characterisation of RIF
Refer Experimental IIIA-1 (pg 201)

2. Analytical Method Development
Refer Experimental IIIA-3.1.1 (pg 202)

3. Formulation Development

3.1. Design of the formulation
To comply with the biopharmaceutical requirement of achieving minimum effective concentration and to elicit the required therapeutic effect in the body, the total dose of formulation (450mg) was divided into two doses: loading dose of 300mg as melt extruded immediate release (IR) pellets and maintenance dose of 150mg as melt extruded tablet (Pund et al., 2011). The selection of polymeric carriers and plasticizers was based on the preformulation studies and calculation of Hansen solubility parameters.

3.2. Formulation of immediate release (IR) pellets
Refer Experimental IIIA-6.1a (pg 207)

3.3. Formulation of RIF ER-I tablets using Klucel matrices (RIF ER SDs I)

3.3.1. Design of extrusion experiments

3.3.1.1. Extrusion trials and processing parameters
Extrusion trials were taken with two viscosity grades of hydroxyl propyl cellulose (avg. molecular weight of MF: 850,000 and LF: 95,000) as the matrix former using the following parameters:

Table IIIB-1: Processing parameters for melt extrusion of RIF ER-I tablets

<table>
<thead>
<tr>
<th>Drug</th>
<th>Batch</th>
<th>Polymer</th>
<th>Drug loading (%)</th>
<th>Plasticizer</th>
<th>Extrusion temperature</th>
<th>Screw speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF</td>
<td>RFX-H1</td>
<td>Klucel MF</td>
<td>50</td>
<td>PG (5%)</td>
<td>85°C</td>
<td>50 rpm</td>
</tr>
<tr>
<td>RFX-H2</td>
<td>Klucel LF</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Development of Extended release formulation of Rifampicin

Approach to tailor drug release: Addition of a pH independent release modifier

<table>
<thead>
<tr>
<th>Approach</th>
<th>Batch</th>
<th>Polymer 1</th>
<th>Polymer 2</th>
<th>Drug loading (%)</th>
<th>Plasticizer</th>
<th>Extrusion temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>RFX-H3</td>
<td>Klucel LF</td>
<td>Klucel MF</td>
<td>50</td>
<td>PG (5%)</td>
<td>85°C</td>
</tr>
</tbody>
</table>

3.3.1.2. *Downstreaming of extrudates*

The extrudates thus obtained were cooled to ambient temperature, cut into tablets equivalent to 150mg RIF and stored in HDPE containers until analysis.

3.3.2. *In vitro dissolution studies*

Dissolution studies were performed according to the following protocol:

*Dissolution apparatus*: USP Type 2 (Paddle) apparatus

*Dissolution medium*: 900 mL, 0.1N HCl for 2 h followed by pH 6.8 buffer + 0.02% ascorbic acid at 37.0 ± 0.5°C

*Speed*: 50 rpm

*Sampling points*: 1, 2, 3, 4, 6, 8, 10, 12, 18, 20, 22 and 24h

*Aliquot withdrawn*: 5 mL

*Method of analysis*: UV-vis spectrophotometry

3.3.3. *Dissolution kinetics*

The mechanism of drug release from the matrices was elucidated by fitting the dissolution data to zero order, first order, Higuchi and Korsmeyer-peppas mathematical models.

3.3.4. *Characterization of RIF ER-I tablets*

Refer methods mentioned in Experimental IB- 4.1.7 (pg 140)

3.4. *Formulation of RIF ER-II minitablets using Kollidon SR matrices*

3.4.1. *Design of extrusion experiments*

3.4.1.1. *Extrusion trials and processing parameters*

Extrusion trials were taken with Kollidon SR as the matrix former using the following parameters:
Development of Extended release formulation of Rifampicin

Table IIIB-2: Processing parameters for melt extrusion of RIF ER-II tablets

<table>
<thead>
<tr>
<th>Batch</th>
<th>Polymer</th>
<th>Drug loading</th>
<th>Plasticizer</th>
<th>Extrusion temperature</th>
<th>Screw speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFX-H4</td>
<td>KSR</td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
<td>50 rpm</td>
</tr>
</tbody>
</table>

Effect of pore formers

Table IIIB-3: Effect of pore formers to tailor release of RIF from KSR matrices

<table>
<thead>
<tr>
<th>Batch</th>
<th>Matrix polymer</th>
<th>Pore former</th>
<th>Drug loading</th>
<th>Plasticizer</th>
<th>Extrusion temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFX-H5</td>
<td>KSR (45%)</td>
<td>-</td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
</tr>
<tr>
<td>RFX-H6</td>
<td>KSR (31.5% = 70% of base polymer weight)</td>
<td>Klucel LF</td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
</tr>
<tr>
<td>RFX-H7</td>
<td>KSR (31.5% = 70% of base polymer weight)</td>
<td>Lutrol F68</td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
</tr>
<tr>
<td>RFX-H8</td>
<td>KSR (31.5% = 70% of base polymer weight)</td>
<td>KVA64</td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
</tr>
<tr>
<td>RFX-H9</td>
<td>Eudragit L100-55</td>
<td></td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
</tr>
</tbody>
</table>

3.4.1.2. Downstreaming of extrudates
The extrudates thus obtained were cooled to ambient temperature, cut into mini tablets equivalent to 150mg RIF, filled in gelatin capsules of size 00 and stored in HDPE containers until analysis.

3.4.2. In vitro dissolution studies
Refer Experimental IIIB-3.3.2 (pg 261)

3.4.3. Characterization of RIF ER-II tablets (RIF ER SDs II)
Refer methods mentioned in Experimental IB- 4.1.7 (pg 140)

3.4.4. Physical characterisation of RIF ER-II tablets
The compressed tablets were evaluated for dimension, hardness and friability using USP methods.
4. Stability studies

The optimized formulation was stored in HDPE bottles and subjected to stability studies as per ICH guidelines for a period of six months at 25 ± 2°C/60 ± 5% RH, 30 ± 2°C/65 ± 5% /RH and 40 ± 2°C/75 ± 5% /RH respectively and monitored for changes in physical attributes, drug content and release pattern.
Results and Discussion IIIB

1. Characterisation of RIF

Refer Results and Discussion IIIA-1 (pg 219)

2. Analytical Method Development

2.1. Ultraviolet-visible Spectrophotometry

Refer Results and Discussion IIIA-3.1.1 (pg 221)

2.2. High Performance Liquid Chromatography

Refer Results and Discussion IIIA-3.2.1A (pg 224)

3. Formulation Development

3.1. Design of the formulation

Oral drug delivery systems are the most preferred route of choice particularly for diseases like TB which requires long term treatment. In order to minimize the concerns associated with current TB therapy, controlled delivery systems providing long term therapeutic effect using a commercially viable technology like melt extrusion were resorted to.

The developed formulation was designed to release RIF in two doses: loading dose of 300 mg as immediate release pellets and maintenance dose of 150 mg as melt extruded tablet.

Multiple unit particulate systems offers myriad of advantages over single unit systems. The formulation advantages include versatility of formulation design, convenient modulation of dosage strengths, tailoring release profiles at different site of GIT, delivery of incompatible drugs in a single dosage form, improved stability and ideal shape for application of film coating due to low surface to volume ratio (Sellasie, 1989; Melia et al., 1994). It also bears certain biopharmaceutical advantages like minimum risk of dose dumping, predictable, reproducible and short gastric residence time leading to less inter- and intra-subject variability, enhanced bioavailability, reduced adverse effects and local irritation in the GIT, reduced food effects on drug absorption and improved patient compliance. In addition to this, there are regulatory advantages like extension of patent protection and product globalization (Roy and Shahiwala, 2009).
Pellets prepared using hot melt extrusion could be immediate release or controlled release depending on the type of matrix polymer used. Unlike the traditional pelletization techniques, number of processing steps are reduced, use of water or solvents are obviated, higher drug loading could be attempted, preliminary coating step is not necessary (Young et al., 2002).

3.2. Formulation of immediate release (IR) pellets (RIF IR SDs)
Refer Results and Discussion IIIA-6.1a (pg 231)

3.3. Formulation of RIF ER-I tablets using Klucel matrices
For extended release tablets, hydroxypropyl cellulose (HPC), a nonionic water soluble cellulose ether was explored as the matrix former. Selection of HPC was based on the preliminary trials conducted where it displayed ease of extrudability at lower temperature (i.e. 85°C-90°C), no die swelling owing to its low molecular weight (about 95,000), ability to accommodate higher drug loading (about 50-60%), high process yield (about 60-65% for a single screw extruder) due to the internal lubricants added to Klucel which ensure easy release from screw and barrel surfaces. Propylene glycol at 5% concentration was used as plasticizer to ensure smooth, uniform melt flow and homogenous end product.

3.3.1. In vitro dissolution studies
Formulation batch RFX-H1 containing medium viscosity grade of HPC (Klucel MF, Pharm) could release only 40% RIF at the end of 24 h whereas RFX-H2 batch containing lower viscosity grade (Klucel LF, Pharm) extended the release upto 17 h (Fig. IIIB-12). The difference in release from both the matrices was due to the difference in their solubilities in the dissolution medium and diffusional pathlength of their swollen phases.
Thus, it was thought that a combination of both viscosity grades (RFX-H3) could optimally control the release rate. The burst release from Klucel LF matrix was reduced by addition of 30% (of base polymer weight) of Klucel MF thereby extending release upto 24 h (Fig. IIB-1).

3.3.2. Dissolution kinetics

Swelling and erosion tendency of the hydrated matrix was reduced due to the presence of the poorly soluble RIF particles within the matrix. RIF release was primarily due to matrix erosion, which occurred at a constant rate resulting in zero order release (Table IIB-4).

Table IIB-4: Drug release kinetics of the optimized batch (RFX-H3)

<table>
<thead>
<tr>
<th>Optimised formula</th>
<th>Zero order $R^2$</th>
<th>First order $R^2$</th>
<th>Higuchi $R^2$</th>
<th>Korsmeyer Peppas $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.995</td>
<td>0.862</td>
<td>0.968</td>
<td>0.961</td>
</tr>
</tbody>
</table>

3.3.3. Characterization of RIF IR pellets and RIF ER-I tablets (RIF IR SDs and RIF ER SDs I)

The pellets and tablets were powdered and sieved through mesh 60 prior to analysis.
3.3.3.1. **Thermal stability analysis by thermogravimetric analysis and UV-spectrophotometry**

Thermogravimetric analysis was carried out as a preliminary check on the thermal stability of the polymers and drug employed. There was no weight loss recorded for RIF up to 195.58°C (Fig. IIIB-2) indicating absence of either solvate or hydrate in the sample. It did not reveal any signs of degradation at the processing temperature thus confirming stability during the residence time of about 3 minutes while extrusion.

TGA analysis of Eudragit EPO revealed that depolymerization reactions occurred at temperature higher than 250°C. The minimum decomposition temperature for Eudragit polymer is 254°C with initial occurrence at double bonds at side chain ends. A second peak between 351°C and 400°C was due to second initiation reaction (Fig. IIIB-2). At these temperatures, initiation was by both end-chain and random chain initiation process. Similarly, HPC did not show any signs of degradation at the processing temperature since loss in weight was recorded beyond 299°C.

![Figure IIIB-2: Thermograms of (a) Pure RIF (b) Plain Eudragit EPO and (c) Plain Klucel LF](image-url)
Furthermore, overlapping UV spectra confirmed the findings of the TGA analysis thereby ascertaining the thermal stability (Fig. IIIB-3).

3.3.3.2. Chemical stability analysis by high performance liquid chromatography
HPLC chromatograms of RIF IR SDs and RIF ER SDs I did not display any degradant peaks or any significant change in the shape, height, area or retention time of the characteristic RIF peak thereby confirming its chemical stability (Fig. IIIB-4).
Table IIIB-5: HPLC parameters to confirm chemical stability of RIF IR SDs and ER SDs I

<table>
<thead>
<tr>
<th>Sample</th>
<th>RT(min)</th>
<th>Area (mAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure RIF</td>
<td>11.356</td>
<td>4816.893</td>
</tr>
<tr>
<td>RIF IR SDs</td>
<td>11.209</td>
<td>4787.902</td>
</tr>
<tr>
<td>RIF ER SDs I</td>
<td>11.262</td>
<td>4827.205</td>
</tr>
</tbody>
</table>

3.3.3.3. Miscibility analysis by differential scanning calorimetry

The DSC curve of pure RIF (Fig. IIIB-5a) exhibited distinct melting endothermic peak at 193.69°C, followed by crystal transformation to form I between 199.96–213.98°C, which is a characteristic of solid-liquid-solid transition and decomposition of form I in the range of 232–265°C. This melting behaviour is typically followed by RIF metastable polymorph form II (Freire, 2009). Thus, in addition to thermal stability and miscibility, DSC measurements could also reveal changes in the polymorphic forms of RIF post extrusion. Thermograms of plain polymers showed absence of Tg owing to their amorphous nature. Fig. IIIB-5c and 5f depict thermograms of physical mixtures of RIF with Eudragit EPO and Klucel with the characteristic endothermic peaks of RIF at 190.26°C and 192.38°C. In case of IR pellets, Eudragit EPO had successfully solubilized RIF indicated by absence of RIF endothermic peak (Fig. IIIB-5d). On the other hand, Klucel was capable of effectively dispersing RIF (partial solubilization) forming a solid dispersion indicated by a small endotherm at 190.85°C (Fig. IIIB-5g). Additionally, DSC data also suggested partial conversion of the crystalline to amorphous forms which was further confirmed by p-XRD studies.
3.3.3.4. Drug crystallinity analysis using powder X-ray Diffraction (p-XRD)

The characteristic peaks of pure RIF form II are depicted in Fig. IIIB-6a at 9.96 and 11.1° 2θ respectively. The peaks in melt extruded pellets appeared to be less intensive compared to pure RIF and additionally the characteristic peaks were seen at 13.6° and 14.35° 2θ which is a characteristic diffraction pattern of RIF polymorph form I (Fig. IIIB-6d). Thus, in agreement with the results of DSC, pXRD confirmed the polymorphic conversion of form II to form I. The XRD pattern of RIF ER SD I exhibited an amorphous halo with reduced sharpness of diffraction patterns compared to pure RIF inferring partial drug solubilisation within the polymeric matrix (Fig. IIIB-6g). These observations further confirm the findings of DSC of partial conversion of crystalline to amorphous form.
Development of Extended release formulation of Rifampicin

Institute of Chemical Technology, Mumbai

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Figure IIIB-6: pXRD diffractograms of (a) Pure RIF (b) Plain Eudragit EPO (c) RIF-EPO PM (d) RIF IR SDs (e) Plain Klucel LF (f) RIF ER PM I and (g) RIF ER SDs I

3.3.3.5. *Fourier transform infrared spectroscopy*

FTIR spectra of RIF form II exhibited a distinct double peak at 1729.8 and 1718 cm\(^{-1}\), broad band over 3560–3100 cm\(^{-1}\) due to absorption by ansa OH group (Fig. IIIB-7a). Eudragit EPO (Fig. IIIB-7b) showed characteristic bands of the ester groups at 1150 - 1190, 1240 and 1270 cm\(^{-1}\), as well as the C = O ester vibration at 1730 cm\(^{-1}\). In addition, CH\(_x\) vibrations could be discerned at 1385, 1450 - 1490 and 2950 cm\(^{-1}\). The absorptions at 2770 and 2820 cm\(^{-1}\) could be assigned to the dimethylamino groups. Although, the spectra of RIF pellet showed a double peak at 1720.2 and 1730.4 cm\(^{-1}\), the polymorphic state of the RIF could not be determined since there could be an interference of the C=O peak of Eudragit EPO at 1730 cm\(^{-1}\). The typical peaks of Klucel LF at 1595 cm\(^{-1}\), double peaks at 2922 and 2980 cm\(^{-1}\) and characteristic double peak of RIF was retained at 1720.2 and 1731.8 cm\(^{-1}\) in tablet formulation confirming the chemically unchanged state (Fig. IIIB-7d and 7e). Moreover, there was broadening of the peaks signifying possibilities of hydrogen bonding between the drug and polymer.
Figure IIIB-7: IR spectra of (a) Pure RIF (b) Plain Eudragit EPO (c) RIF IR SDs (d) Plain Klucel LF and (e) RIF ER SDs I
3.3.3.6. **Surface morphology analysis by scanning electron microscopy**

Pure RIF was seen as uniform rod-like particles (Fig. IIIB-8). RIF IR SDs and RIF ER SDs I exhibited a continuous single phase devoid of long range crystal lattice indicating homogenous molecular level dispersion of RIF in the continuous polymeric structure.

![SEM micrographs of (a) Pure RIF (b) RIF IR SDs and (c) RIF ER SDs I](image)

Figure IIIB-8: SEM micrographs of (a) Pure RIF (b) RIF IR SDs and (c) RIF ER SDs I

**3.4. Formulation of RIF ER-II minitablets using Kollidon SR matrices**

KSR containing spray dried physical mixture of 80% insoluble polyvinyl acetate (PVAc) and 19% soluble polyvinylpyrrolidone (PVP) along with 0.8% of sodium lauryl sulphate as stabilizer and 0.6% of silica as flowability agent, was selected as the matrix former. KSR was selected owing to its pH independent release retarding functionality, excellent extrudability and low hygroscopicity.

**3.4.1. In vitro dissolution studies**

KSR hot-melt extruded mini tablets maintained their geometric shape throughout the dissolution study which could be ascribed to the plastic PVAc constituting about 80% of KSR. Additionally, the strongly water soluble PVP component solubilised in the
dissolution medium leaving pores for entry of solvent molecules to dissolve the drug causing a diffusion controlled release mechanism.

From Fig. IIIB-9, it is evident that KSR alone could release only 11.434% of RIF at the end of 24 h of which 8.336% was released in the acid stage. This behaviour could be due to high polymer concentration (~50%) which produced a coherent matrix thus sustaining RIF release. Additionally, poor solubility of RIF above pH 5.5 led to lower porosity and higher tortuosity of the matrix thereby further retarding RIF diffusion.

![Dissolution Profile of RIF in Kollidon® SR Matrix](image)

*Data is expressed as mean ± SD, n = 3

Figure IIIB-9: *In vitro* dissolution of RIF ER-II minitablets using KSR matrix only

Additionally, there could be a possibility of strong binding of RIF on KSR matrix resulting in incomplete RIF release. At higher drug loading, RIF release could be explained by pores created by the dissolved RIF particles in addition to the pores created by the water soluble PVP component of KSR.

Keeping above observations in view, there was a need for addition of pore formers to improve drug release in the buffer stage. Thus, Klucel LF, Lutrol F68, Kollidon VA64 and Eudragit L100-55 were added at 30% concentration to the KSR matrix to facilitate RIF release. The extrusion temperature was kept constant all throughout since change in temperature could cause changes in RIF solubilization in the matrix and hence a variable release.
The release of RIF was enhanced in the following order: Klucel LF < Lutrol F68 < Kollidon VA64 < Eudragit L100-55. Inclusion of pore formers improved RIF dissolution in the gastric phase from 8.336% to Eudragit L100-55 displaying maximum release of 34.7%, followed by Kollidon VA64 (29.705%), Lutrol F68 (25.275%) and Klucel LF (19.156%).

Klucel LF, a non-ionic, hydrophilic, surface active, swellable polymer leached into the dissolution medium creating a more porous matrix thus enhancing release of RIF. However, as hydration time progresses, the pores are blocked due to swollen phase of Klucel LF impeding further liquid entry and restricting RIF release.

Lutrol F68 (HLB value: 29) belonging to the category of solubilizer, surface active/wetting agent (surface tension: 50 dynes/cm for 0.1% aq. solution at 25°C) and plasticizer, lowered the interfacial tension between RIF and dissolution medium thereby increasing the wettability of RIF and KSR. The solubility of Lutrol F68 in water is believed to be due to hydrogen bonding interactions of ether oxygen of poly ethylene oxide block with water molecules. Owing to its high molecular weight (avg mol.wt: 8600) compared to classical plasticizers like PEG or TEC, they are not readily soluble in many polymers and often form crystalline regions within the solid dispersion thus limiting solubilization. The mechanism of release exhibited by both the above mentioned pore former was primarily diffusion and erosion to a lesser extent.
Kollidon VA64, a vinylpyrrolidone-vinyl acetate (60:40) copolymer is used as a solubilizer. On extrusion, KVA64 might have improved the solubility of RIF leading to its molecular level dispersion within the matrix. On hydration, RIF-VA64 SDs dispersed within the KSR matrix began to solubilize thereby eroding the matrix and facilitating RIF release in buffer stage.

Eudragit L100-55, an anionic copolymer based on methacrylic acid and ethyl acrylate (1:1) is soluble at pH 5.5 and above. At low pH, Eudragit L100-55 is insoluble and the release of RIF was governed by diffusion whereas at higher pH the matrix becomes soluble and release of RIF was governed by solubilization of the Eudragit L100-55. Additionally, it also weakened interaction between RIF and KSR due to anionic functional groups on it. In essence, addition of Eudragit L100-55 to KSR matrix not only imparted gastric protection to RIF in acid phase but also aided its release in buffer stage by eroding the matrix.

To support the dissolution findings, weights of minitablets were monitored periodically and difference in the values was recorded. There was a 30% and 47.778% reduction in weight after 24 h of dissolution for minitablets containing L100-55 and KVA64 respectively thereby confirming the predominant erosion mechanism (Fig. IIB-10 and 11). On the contrary, there was no significant change in the weight of minitablets containing Klucel LF and Lutrol F68.
However, high solubility of RIF from the mini tablets in the acid media displayed a burst release which was in addition to release from RIF IR pellets (loading dose). Thus, it was further decided to incorporate the loading dose (IR RIF: 300mg) as KSR minitablets (total dose of minitablets: 450mg).
RIF minitablets equivalent to 450mg RIF was subjected to *in vitro* dissolution using the same protocol.

![Comparative Dissolution Profiles of KSR Mini Tablets](image)

**Figure IIIB-12:** Effect of pore formers on dissolution of RIF ER-II minitablets (450mg)

![Extended Release RIF Capsule](image)

**Figure IIIB-13:** An extended release RIF capsule containing minitablets (450mg)

At high dose, Eudragit L100-55 and KVA 64 successfully sustained release over 24 h with a loading dose of about 68.7% i.e. 309.15mg (by KVA64) and 63.36% i.e. 285.12mg (by Eudragit L100-55) of RIF within 2 h thereby satisfying the biopharmaceutical criteria.

Thus, formulations containing Eudragit L100-55 (RIF ER SDs II) and Kollidon VA64 (RIF ER SDs III) were further subjected to characterisation and stability studies.
3.4.2. Characterization of RIF ER-II minitablets (RIF ER SDs II and III)

3.4.2.1. Thermal stability analysis by thermogravimetric analysis and UV-spectrophotometry

Refer Results and Discussion IIIB-3.3.3.1 (pg 267) for TGA analysis

Figure IIIB-14: UV-vis spectras of RIF in minitablets containing (a) Pure RIF (b) Plain KSR (c) Eudragit L100-55 (d) KVA64 (e) Klucel LF and (f) Lutrol F68

The overlapping UV spectral pattern confirmed the preliminary thermal stability of RIF post extrusion (Fig. IIIB-14).

3.4.2.2. Chemical stability analysis by high performance liquid chromatography

HPLC chromatograms of RIF ER SDs II and RIF ER SDs III did not display any degradant peaks or any significant change in the shape, height, area or retention time of the characteristic RIF peak thereby confirming its chemical stability (Fig. IIIB-15).

Table IIIB-6: HPLC parameters to confirm chemical stability of RIF ER SDs II and III

<table>
<thead>
<tr>
<th>Sample</th>
<th>RT(min)</th>
<th>Area (mAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure RIF</td>
<td>11.460</td>
<td>3863.585</td>
</tr>
<tr>
<td>RIF ER SDs II</td>
<td>11.313</td>
<td>3979.758</td>
</tr>
<tr>
<td>RIF ER SDs III</td>
<td>11.310</td>
<td>3948.751</td>
</tr>
</tbody>
</table>
3.4.2.3. Miscibility analysis by differential scanning calorimetry

Plain Eudragit L100-55 displayed a broad endotherm between 176-220°C whereas KSR and KVA64 showed absence of Tg owing to their amorphous nature as indicated in Fig. IIIB-16c, 16b and 16f. Fig. IIIB-16d and 16g represents thermograms of physical mixtures of RIF and KSR with Eudragit L100-55 and KVA64 with the characteristic endothermic peaks of RIF at 193.61°C and 193.58°C respectively. Both RIF ER SDs II and III underwent direct decomposition indicated by an exothermic peak between 251–270°C which is the characteristic melting behaviour of RIF polymorph form I (Fig. IIIB-16e and 16h). This polymorphic conversion was further confirmed by p-XRD studies.
3.4.2.4. Drug crystallinity analysis using powder X-ray Diffraction (p-XRD)

The XRD patterns of both RIF ER SDs II and III resembled that of polymorphic form I with the characteristic peaks at 13.68 and 14.81° 2θ for RIF ER SDs II and at 12.42 and 13.51° 2θ for RIF ER SDs III (Fig. IIIB-17b and 17c). These findings were in concordance with the inference of DSC results thereby confirming the change from RIF form II to form I.
Figure IIIB-17: pXRD diffractograms of (a) Pure RIF (b) RIF ER SDs II and (c) RIF ER SDs III
3.4.2.5. Fourier transform infrared spectroscopy

FTIR was used as a qualitative tool to identify the physical form as well as interactions of the carrier and pore formers with RIF.

Figure IIIB-18: IR spectras of (a) Pure RIF (b) Plain Kollidon SR (c) RIF ER PM II (d) RIF ER SDs II (e) RIF ER PM III and (f) RIF ER SDs III
In RIF, all the functional groups that can be involved in H-bonding are bonded intramolecularly that shows differences in ansa OH, furanonic, acetyl and amide C=O frequencies. The possible intramolecular bonding reported in the IR spectrum of RIF in CDCl$_3$ solution are C21 hydroxyl is H-bonded to the C23 hydroxyl which in turn is H-bonded to C25 acetyl, C8 hydroxyl is bonded to C1 hydroxyl which in turn is bonded to the amide carbonyl, C4 hydroxyl is bonded to furanone carbonyl and the amide NH to imine nitrogen of the substituent at C3 (Pelizza, 1977). In form I, C23 hydroxyl is not H-bonded to the acetyl group while all other intramolecular hydrogen bonds are operative whereas in form II, C4 hydroxyl is not bonded to furanone carbonyl and C1 hydroxyl is not bonded to amide carbonyl. In amorphous state, acetyl group exists in two different conformations that prevent the formation of ordered crystalline state. Due to above mentioned differences, in the hydrogen bonding, form II clearly shows characteristic double peaks at 1712 and 1734 cm$^{-1}$ due to acetyl and furanone C=O, respectively, whereas form I and amorphous shows only a single peak at 1725 cm$^{-1}$. Further, absorption due to ansa OH gives sharp band at 3481 cm$^{-1}$ in case of form I, whereas for form II and amorphous it is a broad band over 3565–3150 cm$^{-1}$ (Agrawal, 2004).

In case of RIF ER SDs II and III, the characteristic RIF form II double peak at 1718 and 1729.8 cm$^{-1}$ was absent. PM II (Fig. IIIB-18c and 18e) exhibited a double peak at 1713.64, 1724.04 cm$^{-1}$ (RIF form II) and 1590.05 (KSR). For SDs II, there was a single broad peak at 1729.75 cm$^{-1}$ which could be contributed by Eudragit L100-55 (1729.85 cm$^{-1}$) and Kollidon SR (1736.75 cm$^{-1}$). Similarly, SDs III displayed peaks at 1593.47 cm$^{-1}$ (KSR) and 1727.2 cm$^{-1}$ (KVA64). From these observations it could be inferred that there might be a possibility that the aforesaid peaks would have interfered with RIF form I peak at 1725 cm$^{-1}$ thereby hampering the prominence of the same. Hence, IR studies could not assist much in monitoring the change of polymorphic forms.

### 3.4.2.6. Surface morphology analysis by scanning electron microscopy

Pure RIF was seen as uniform rod like particles and plain KSR appeared as spherical particles. The RIF ER SDs exhibited a continuous single phase devoid of long range crystal lattice indicating homogenous molecular level dispersion of RIF in the continuous polymeric structure (Fig. IIIB-19).
Development of Extended release formulation of Rifampicin

3.4.3. Physical characterisation of RIF minitablets

Table IIIB-7: Physical characterisation of RIF minitablets

<table>
<thead>
<tr>
<th>Minitablets</th>
<th>Dimension</th>
<th>Hardness (kg/cm²)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (mm)</td>
<td>Thickness (mm)</td>
<td></td>
</tr>
<tr>
<td>RIF ER SDs II</td>
<td>3.733 ± 0.1419</td>
<td>2.379 ± 0.234</td>
<td>4-5</td>
</tr>
<tr>
<td>RIF ER SDs III</td>
<td>4.238 ± 0.1858</td>
<td>2.513 ± 0.230</td>
<td>4-5</td>
</tr>
</tbody>
</table>

4. Stability studies

The optimised formulations were found to be stable for six months as per ICH guidelines with no significant changes in dissolution behaviour (Fig. IIIB-20, 21 and 22), drug content (Table IIIB-8) and physical stability.

Table IIIB-8: Effect of storage on content of RIF in ER SDs
## Development of Extended release formulation of Rifampicin

### Stability conditions

<table>
<thead>
<tr>
<th>Duration</th>
<th>25°C ± 2°C/RH 60 ± 5%</th>
<th>30°C ± 2°C/RH 65 ± 5%</th>
<th>40°C ± 2°C/RH 75 ± 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug content (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIF ER SDs I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 day</td>
<td>100.211 ± 0.912</td>
<td>99.745 ± 0.324</td>
<td>103.267 ± 0.142</td>
</tr>
<tr>
<td>1 month</td>
<td>99.841 ± 1.654</td>
<td>98.452 ± 4.125</td>
<td>101.145 ± 2.314</td>
</tr>
<tr>
<td>3 months</td>
<td>98.113 ± 2.301</td>
<td>98.024 ± 1.236</td>
<td>99.127 ± 3.140</td>
</tr>
<tr>
<td>6 months</td>
<td>100.021 ± 3.654</td>
<td>97.362 ± 2.541</td>
<td>97.185 ± 2.396</td>
</tr>
<tr>
<td>RIF ER SDs II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 day</td>
<td>99.345 ± 2.671</td>
<td>102.471 ± 2.478</td>
<td>99.687 ± 1.455</td>
</tr>
<tr>
<td>1 month</td>
<td>99.128 ± 1.653</td>
<td>101.251 ± 1.654</td>
<td>99.122 ± 2.354</td>
</tr>
<tr>
<td>3 months</td>
<td>98.487 ± 0.240</td>
<td>100.487 ± 3.412</td>
<td>98.487 ± 0.364</td>
</tr>
<tr>
<td>6 months</td>
<td>98.004 ± 4.234</td>
<td>95.421 ± 3.632</td>
<td>96.471 ± 2.006</td>
</tr>
<tr>
<td>RIF ER SDs III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 day</td>
<td>106.124 ± 1.654</td>
<td>101.691 ± 0.487</td>
<td>98.241 ± 1.654</td>
</tr>
<tr>
<td>1 month</td>
<td>103.541 ± 2.410</td>
<td>100.354 ± 2.547</td>
<td>99.487 ± 3.875</td>
</tr>
<tr>
<td>3 months</td>
<td>102.472 ± 1.568</td>
<td>99.487 ± 3.541</td>
<td>98.420 ± 3.875</td>
</tr>
<tr>
<td>6 months</td>
<td>103.661 ± 3.244</td>
<td>98.189 ± 2.541</td>
<td>94.235 ± 4.225</td>
</tr>
</tbody>
</table>

*Data is expressed as mean ± SD, n = 3

### In vitro dissolution (as given below)

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(a)

(b)

(c)
Figure IIIB-20: Effect of storage on dissolution behaviour of RIF ER SD I formulation at (a) 25°C/60% RH (b) 30°C/65% RH and (c) 40°C/75% RH.

Figure IIIB-21: Effect of storage on dissolution behaviour of RIF ER SD II formulation at (a) 25°C/60% RH (b) 30°C/65% RH and (c) 40°C/75% RH.
Figure IIIB-22: Effect of storage on dissolution behaviour of RIF ER SD III formulation at (a) 25°C/60% RH (b) 30°C/65% RH and (c) 40°C/75% RH
Conclusion IIIB

- Controlled delivery systems possess potential advantages over the conventional multidose delivery systems, particularly for long-term therapeutic effect and for the treatment of chronic diseases like TB.

- Extended release stable RIF formulations were successfully developed to overcome its concentration dependent degradation. using HME by both approaches:
  - Approach I: Formulation with a loading dose of RIF as pellets and maintenance dose as a tablet. The tablets prepared using hydrophilic release retardant like hydroxypropyl cellulose gave a zero order release profile.
  - Approach II: Minitablets (filled in capsules) were prepared using plastic matrix former like Kollidon SR. Moreover, addition of pore formers to these matrices gave a bimodal release with initial loading dose of 300mg followed by maintenance dose of 150mg.

- With regards to scalability, approach II appears to be a better alternative due to less number of unit operations involved, better scope of tailoring drug release by varying the concentrations of pore formers and above all the advantages of multiparticulate delivery system over conventional systems.