## Literature survey/ Prior art IIIC

<table>
<thead>
<tr>
<th>Technique used</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous or emulsion-based salting-out</td>
<td>INH loaded polymeric nanosystem using Eudragit® L100-55 and zinc sulphate as the crosslinking agent (Toit et al., 2008)</td>
</tr>
<tr>
<td>Direct compression</td>
<td>Matrix tablets using different proportion and combination of guar gum and carbopol, tragacanth gum and PEG 6000 (Suresh et al., 2011)</td>
</tr>
<tr>
<td>Double emulsification solvent evaporation</td>
<td>Porous/ non-porous/hardened biodegradable Poly (DL-lactide-co-glycolide) microparticles as a single injectible dose (Dutt and Khuller, 2001)</td>
</tr>
<tr>
<td>Lyophilization</td>
<td>Biodegradable polymeric foams (prior to compaction and extrusion) of low density poly(DL-lactide-co-glycolide) (Hsu et al., 1996)</td>
</tr>
<tr>
<td>Microencapsulation</td>
<td>Microparticles of INH by microencapsulation using polyelectrolyte complex of gelatin A and NaCMC, glutaraldehyde (crosslinking agent), water (solvent) and sunflower oil (emulsion medium) (Devi and Maji, 2009)</td>
</tr>
<tr>
<td>Modified emulsification</td>
<td>INH spherical microspheres were developed by a modified emulsification method followed by cross-linking with calcium chloride using sodium alginate as the hydrophilic carrier (Rastogi et al., 2007)</td>
</tr>
<tr>
<td>Spray drying</td>
<td>Microspheres of INH for pulmonary delivery in a crosslinked chitosan matrix using tripolyphosphate as ionic cross linker (Kundawala et al., 2011)</td>
</tr>
<tr>
<td>Wet non-aqueous granulation</td>
<td>Hydrophilic matrix formulation of INH (HPMC.K100M, K4M, K15M and K100LV) (Hiremath and Saha, 2008)</td>
</tr>
</tbody>
</table>
Rationale IIIC

- Isoniazid, an antimycobacterial agent for first line therapy of tuberculosis has been classified as a BCS class I drug i.e. highly soluble and highly permeable.

- Pharmacokinetic considerations

  i. INH has pronounced absorption from all the three segments of the small intestine (Mariappan and Singh, 2003).

  ii. It is characterized by a short half life of about 0.5-1.6 h (fast acetylators) and 2-5 h (for slow acetylators).

  iii. It undergoes pre-systemic (first pass) metabolism in the wall of the small intestine and liver mainly by acetylation and dehydrazination. The rate of acetylation is genetically determined and subject to individual variation resulting in concentrations in the plasma of rapid acetylators which are half those in slow acetylators after normal dose of 300 mg of INH (Eidus and Hodgkin, 1975). This results in subtherapeutic concentrations of INH in the blood, which leads to treatment failure and also encourages INH resistant strains of M.tb.

  iv. Long-term continuous therapy with INH leads to hepatotoxicity and peripheral neuritis which may lead to discontinuation of the therapy due to lack of patient compliance.

- After intravenous administration of INH, there was no significant difference in the peak plasma concentrations of rapid and slow acetylators thus indorsing the development of ER formulations of INH to optimize the blood levels in the rapid acetylators (Ellard, 1972).

Objective

The objective in designing a sustained release tablet form was to attain sustained blood concentration in fast acetylators similar to those produced by ordinary INH tablets in slow acetylators during chemotherapy.
Development of Extended release formulation of Isoniazid

Experimental IIIC

1. Characterisation of INH
Refer Experimental IIA-2 (pg 201)

2. Analytical Method Development
2.1. Ultraviolet-visible Spectrophotometry
Refer Experimental IIA-3.1.2 (pg 202)

2.2. Reverse Phase High Performance Liquid Chromatography
Refer Experimental IIA-3.2.3B (pg 204)

3. Formulation Development
3.1. Formulation of INH extended release melt extruded tablets (INH ER SDs)
3.1.1. Design of extrusion experiments
3.1.1.1. Extrusion trials and processing parameters
Extrusion trials were taken with three matrix formers belonging to different classes: Klucel, Eudragit RSPO and Kollidon SR using the following parameters:

<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>INH</td>
<td>INX-1</td>
<td>Klucel MF</td>
<td>50</td>
<td>PG (5%)</td>
<td>85°C</td>
<td>50 rpm</td>
</tr>
<tr>
<td></td>
<td>INX-2</td>
<td>Eudragit RSPO</td>
<td>50</td>
<td>TEC (5%)</td>
<td>105°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>INX-3</td>
<td>Kollidon SR</td>
<td>50</td>
<td>TEC (5%)</td>
<td>95°C</td>
<td></td>
</tr>
</tbody>
</table>

Approach to tailor drug release: Addition of a lipophilic release modifier

<table>
<thead>
<tr>
<th>Batch</th>
<th>Matrix polymer</th>
<th>Release modifier</th>
<th>Drug loading (%)</th>
<th>Plasticizer</th>
<th>Extrusion temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>INX-4</td>
<td>Klucel MF</td>
<td>42.75% (95%*)</td>
<td>2.25% (5%*)</td>
<td>PG (5%)</td>
<td>85°C</td>
</tr>
<tr>
<td>INX-5</td>
<td>40.5% (90%*)</td>
<td>4.5% (10%*)</td>
<td>50</td>
<td>PG (5%)</td>
<td>85°C</td>
</tr>
<tr>
<td>INX-6</td>
<td>38.25% (85%*)</td>
<td>6.75% (15%*)</td>
<td>50</td>
<td>PG (5%)</td>
<td>85°C</td>
</tr>
<tr>
<td>INX-7</td>
<td>36% (80%*)</td>
<td>9% (20%*)</td>
<td>50</td>
<td>PG (5%)</td>
<td>85°C</td>
</tr>
</tbody>
</table>

*Percentage mentioned is with respect to base polymer weight
3.1.1.2. Downstreaming of extrudates
The extrudates thus obtained were cooled to ambient temperature, cut into tablets equivalent to 300mg INH and stored in HDPE containers until analysis.

3.1.2. 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 phosphate buffer at 37.0 ± 0.5°C
- **Speed**: 50 rpm
- **Sampling points**: 1, 2, 3, 4, 6, 8, 10, 12, 18, 20, 22h
- **Aliquot withdrawn**: 5 mL
- **Method of analysis**: UV-vis spectrophotometry

3.1.3. 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.

3.1.4. Characterization of the INH ER SDs
Refer methods mentioned in Experimental IB- 4.1.7 (pg 140)

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 IIIC

1. Characterisation of INH
Refer Results and Discussion IIIA-2 (pg 220)

2. Analytical Method Development
Refer Results and Discussion IIIA-3.1.2 (pg 223) and 3.2.3B (pg 226)

3. Formulation Development

3.1. Formulation of INH ER melt extruded tablet
It is widely suggested that to obtain the desired therapeutic effect, INH ER formulations should be designed such that it releases approximately 37% of INH initially as a loading dose and 63% as a maintenance dose in a controlled manner (Eidus and Hodgkin, 1975). In line with the above suggestion, ER formulation was developed initially using three different classes of polymers: Kollidon SR (PVAc-PVP mixture), Klucel MF (hydroxypropyl cellulose) and Eudragit RSPO (copolymer of ethyl acrylate, methyl methacrylate and low content of methacrylic acid ester with quaternary ammonium groups).

3.1.1. In vitro dissolution studies
As seen from fig. IIIC-1, INX-1 could sustain INH release only upto 5 h owing to hydrophilic swellable matrix former Klucel MF and intrinsic hydrophilicity of INH. INX-2 displayed extended release upto 14 h due to pH independent swelling and lower permeability of Eudragit RSPO whereas INX-3 showed incomplete release of about 38.669% owing to the presence of PVAc (plastic material) in KSR which produces a coherent matrix thereby prolonging INH release.

![Comparative Dissolution Profiles of Extended Release INH](image-url)

**Figure IIIC-1:** *In vitro* dissolution of INH ER tablets
Thus, there was a need to tailor INH release by addition of suitable lipophilic modifiers to the matrix. Klucel MF was selected as the matrix former due to its dimensional stability post extrusion. Stearic acid was selected as the release modifier due to its ability to produce waxy hydrophobic matrices, thermal stability and higher process yield (about 75%) due to its lubricating property.

Figure IIIC-2: Effect of varying concentration of stearic acid on release of INH ER tablets

As anticipated, addition of increasing amount of stearic acid from 5 to 20%, delayed wetting of matrix thereby optimizing the initial release and sustain release of INH upto 18-22 h. (Figure IIIC-2). As concentration of stearic acid increased from 5 to 20%, the loading release decreased from 50.351% to 30.691% with optimum loading displayed by 15% and 20% stearic acid between 37.448% - 30.691%. The loading INH dose could achieve initial amount required to elicit necessary therapeutic action and the controlled release component compensated for the decreased half life in fast acetylators. Due to extended release profile of INX-7 upto 24 h, the batch was selected for further analysis. Melt extrusion which is a single step melt granulation process caused stearic acid to melt, redistribute, coat INH and Klucel MF forming a network structure.

3.1.2. Dissolution kinetics

The mechanism of release by optimised formulation was best expressed by Higuchi’s equation as the plots showed high linearity with a ‘r’ value of 0.982 indicating diffusion mediated transport. For definitive mechanistic conclusion, the data were fit into Korsmeyer Peppas’s equation. The formulation showed good linearity (R²: 
0.9525), with slope (n) value of 1.0545 indicating a case II transport (swelling controlled mechanism) where relaxation of the drug molecules occurring upon water imbibition into the system is the rate controlling step. Water acts as a plasticizer and decreases the Tg of the polymer. Once the Tg equals temperature of the system, the polymer chains undergo transfer from glassy to rubbery state, with increasing mobility of the drug molecules and volume expansion (Siepmann and Peppas, 2001).

Table IIIC-2: Drug release kinetics of the optimized batch (INX-7)

<table>
<thead>
<tr>
<th>Optimised formula</th>
<th>Zero order</th>
<th></th>
<th>First order</th>
<th></th>
<th>Higuchi</th>
<th></th>
<th>Korsmeyer Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td></td>
<td>$R^2$</td>
<td></td>
<td>$R^2$</td>
<td></td>
<td>$R^2$</td>
</tr>
<tr>
<td>Optimised formula</td>
<td>0.9239</td>
<td></td>
<td>0.8027</td>
<td></td>
<td>0.9822</td>
<td></td>
<td>0.9525</td>
</tr>
<tr>
<td></td>
<td>1.0545</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

3.1.3. Characterization of the INH ER SDs

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

For Thermal stability by TGA, refer Results and Discussion IIIA-6.1b.3.1. (pg 238) (Fig. IIIA-24). Preliminary thermal stability was ascertained by overlapping UV spectras of pure INH and INH ER SDs.

![Figure IIIC-3: UV-vis spectras of (a) Pure INH and (b) INH ER SDs](image-url)
3.1.3.2. Chemical stability analysis by high performance liquid chromatography

HPLC chromatograms of pure INH and INH ER SDs did not display any degradant peaks or any significant change in the shape, height, area or retention time of the characteristic INH peak thereby confirming its chemical stability (Figure IIIC-4).

![Figure IIIC-4: HPLC Chromatograms of (a) Pure INH and (b) INH ER SDs](image)

**Table IIIC-3: HPLC parameters to confirm chemical stability of INH ER SDs**

<table>
<thead>
<tr>
<th>Sample</th>
<th>RT(min)</th>
<th>Area (mAU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure INH</td>
<td>2.772</td>
<td>2624.173</td>
</tr>
<tr>
<td>INH ER SDs</td>
<td>2.778</td>
<td>2675.734</td>
</tr>
</tbody>
</table>

3.1.3.3. Miscibility analysis by differential scanning calorimetry

The DSC thermograms of pure INH and stearic acid displayed single melting endotherm at 175.36°C and 61.16°C respectively. Klucel MF showed absence of Tg owing to their amorphous nature.

The physical mixture of the blend showed characteristic peaks of both INH and stearic acid whereas SDs showed reduction in the peak area and height of INH. This implies reduction in crystallinity of INH and its miscibility within the Klucel MF matrix due to the melt extrusion process.
3.1.3.4. Powder X-ray Diffraction (p-XRD), Fourier transform infrared spectroscopy, Surface morphology analysis: Analysis could not be performed as the extrudates could not be milled (even after freezing) due to high plasticity of Klucel MF.

4. Stability studies
The optimised formulation was found to be stable for three months as per ICH guidelines with no significant changes in dissolution behaviour (figure IIIC-5) and drug content (table IIIC-4). However, at 40°C/75% RH, the release was incomplete which could be attributed to sintering effect on the stearic acid containing matrices over storage. In that case, INX-6, could have been selected because the sintering effect would have compensated for the earlier release of INH till 18 h thereby retarding release further till 24 h.

Table IIIC-4: Effect of storage on content of INH
Development of Extended release formulation of Isoniazid

<table>
<thead>
<tr>
<th>Duration</th>
<th>Stability conditions</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C ± 2°C/RH 60 ± 5%</td>
<td>97.263 ± 2.547</td>
</tr>
<tr>
<td></td>
<td>30°C ± 2°C/RH 65 ± 5%</td>
<td>98.478 ± 0.257</td>
</tr>
<tr>
<td></td>
<td>40°C ± 2°C/RH 75 ± 5%</td>
<td>97.775 ± 1.988</td>
</tr>
</tbody>
</table>

0 day  | 97.025 ± 1.487  | 98.367 ± 1.954  | 96.358 ± 3.635  |
1 month | 97.007 ± 1.963  | 98.221 ± 1.255  | 96.247 ± 5.267  |
2 months| 96.978 ± 3.875  | 98.116 ± 2.648  | 96.105 ± 4.551  |
3 months|                 |                 |                 |

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

In vitro dissolution (as given below)

Figure IIIC-6: Effect of storage on dissolution behaviour of INH ER SDs formulation at (a) 25°C/60% RH (b) 30°C/65% RH and (c) 40°C/75% RH
Conclusion IIIC

- Extended release tablets of INH were successfully developed using melt extrusion.
- A blend of hydrophilic and lipophilic retardants proved to be excellent matrix former for highly soluble drug like INH. In addition, it could successfully release the required loading dose in the initial hour to elicit the therapeutic effect. Moreover, stearic acid also acted as a lubricant thereby improving the yield of the process.
- The formulation was found to be stable for 3 months