CHAPTER 2
INTRODUCTION
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2. Introduction

2.1 Introduction to Tuberculosis (TB)

2.1.1 Pathogenesis

Tuberculosis is caused by the tubercle bacillus i.e. *Mycobacterium tuberculosis* and/or *Mycobacterium bovis* (Selwyn, 1989; Daley, 1992).

2.1.2 Type

Depending on the severity of infection, TB is classified as primary and secondary tuberculosis.

*Primary infection*: It occurs on exposure to tubercle bacilli.

*Secondary TB*: It may result from reactivation of dormant lesion or quiescent lesion or in some cases, it may follow reinfection of a person who is already hypersensitive (tuberculin positive) as a result of an earlier primary infection. Tuberculosis is also classified on the basis of the site of infection as Pulmonary TB and extrapulmonary TB.

2.1.3 Symptoms

The symptoms of pulmonary TB include productive cough, loss of appetite, weight loss, fatiguability, fever, chest pain, night sweat, haemoptysis, breathlessness and night sweats (Mandell, 1980; Mukherjee, 1994).

2.1.4 Clinical Diagnosis

A number of tests, ranging from acid-fast smears to complex serologic studies, are available for identifying TB infections. Patient evaluation includes medical history, sputum examination, acid fast bacilli test and X-ray examination. Extrapulmonary TB is suspected by urine examination, cerebrospinal fluid, and pleural fluid. Certain modern techniques such as amplified Mycobacterium tuberculosis direct test (gene probe), polymerase chain reaction, firefly luciferase, culture techniques, serologic assays, tuberculin skin test and BCG vaccine. Skin testing and imaging techniques are also used for the diagnosis (WHO, 1997; Shishoo, 2001).
2. Introduction

2.1 Treatment

There are many different possible anti-TB treatment regimes. The WHO and IUATLD recommend standardized TB treatment regimens (Mandell, 1980; Mukherjee, 1994). The recommended doses for anti-TB drugs are as shown in Table-2.1.

<table>
<thead>
<tr>
<th>Anti-TB drugs</th>
<th>Mode of action</th>
<th>Recommended daily dose (mg/kg)</th>
<th>Daily</th>
<th>Intermittent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 x per week</td>
</tr>
<tr>
<td>Isoniazid (H)</td>
<td>Bactericidal</td>
<td>5 (4-6)</td>
<td>10 (8-12)</td>
<td>15 (13-17)</td>
</tr>
<tr>
<td>Rifampicin (R)</td>
<td>Bactericidal</td>
<td>10 (8-12)</td>
<td>10 (8-12)</td>
<td>10 (8-12)</td>
</tr>
<tr>
<td>Pyrazinamide (P)</td>
<td>Bactericidal</td>
<td>25 (20-30)</td>
<td>35 (30-40)</td>
<td>50 (40-60)</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>Bactericidal</td>
<td>15 (12-18)</td>
<td>30 (25-35)</td>
<td>45 (40-50)</td>
</tr>
<tr>
<td>Ethambutol (E)</td>
<td>Bacteriostatic</td>
<td>15 (15-20)</td>
<td>15 (12-18)</td>
<td>15 (12-18)</td>
</tr>
<tr>
<td>Thioacetzone (T)</td>
<td>Bacteriostatic</td>
<td>2.5</td>
<td>Not applicable</td>
<td></td>
</tr>
</tbody>
</table>

* WHO does not generally recommend twice weekly regimens. If a patient receiving a twice-weekly regimen misses a dose of tablets, this missed dose represents a bigger fraction of the total number of treatment doses than if the patient were receiving a thrice weekly or daily regimen. There is therefore a bigger risk of treatment failure. The parenthesis shows the range of dose.

WHO and IUATLD recommend the use of FDC formulations of the essential antituberculosis drugs as one further step to ensure adequate treatment of patients (Fourie, 1999; Rouhi, 2002).

2.1.6 Drug resistance

Emergence of drug resistance presents a major threat to the future success of TB control. Microbial resistance can be of primary, secondary, or multidrug resistance type. Primary
resistance occurs in patients who are not known to have previous antimycobacterial treatment. Secondary resistance occurs in patients who have been treated in the past. Multi-drug resistant TB is a state of TB caused by Mycobacterium tuberculosis that exhibits resistance to at least isoniazid and rifampicin with or without resistance to others (Spratt, 1994). The incidence rate of MDR-TB associated with death is very high (Dooley, 1992). Table-2.2 shows the standard daily treatment for tuberculosis (Fourie, 1999).

Table-2.2 Standard daily treatment for tuberculosis.

<table>
<thead>
<tr>
<th>TB treatment Category</th>
<th>TB Patient’s Characteristics</th>
<th>Alternative TB treatment regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>New sputum smear-positive pulmonary tuberculosis; new smear-negative patient with extensive parenchymal involvement; new cases of severe forms of extrapulmonary TB.</td>
<td>2 EHRZ (SHRZ) 2 EHRZ (SHRZ) 2 EHRZ (SHRZ)</td>
</tr>
<tr>
<td>2.</td>
<td>Sputum smear-positive; relapse; treatment failure; treatment after interruption</td>
<td>2 SHRZE/1HRZE 2 SHRZE/1HRZE</td>
</tr>
<tr>
<td>3.</td>
<td>New smear-negative patient (other than in category 1); new less severe forms of extrapulmonary TB</td>
<td>2 HRZ 2 HRZ 2 HRZ</td>
</tr>
<tr>
<td>4.</td>
<td>Chronic case (sputum-positive after supervised re-treatment)</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

Note: A 7 month continuation phase with daily isoniazid and rifampicin (7 HR) for category 1 patients has been recommended for TB meningitis, military TB, spinal TB with neurological signs. E = ethambutol, H = isoniazid, R = rifampicin, Z = pyrazinamide, S = streptomycin. The number before a phase is the duration of that phase in months. A number after a letter is the number of doses of that drug per week.
2.2 Introduction to Fixed Dose Combination (FDC)

WHO and IUATLD advocate the replacement of single-drug preparations by FDC tablets as the primary treatment for TB, since 1994. WHO states that the use of FDC preparations will simplify the prescription and drug ingestion, rationalize drug supply, and all these advantages will ultimately help to prevent drug resistance. The use of FDCs would simplify not only the prescription but also the operational procedures of supply, order, delivery distribution, and storage. Patient compliance is one major advantage of these FDCs.

In 1999, the WHO model list of essential drugs (EDL) was updated to include four FDCs, containing 150 mg rifampicin, 75 mg isoniazid, 400 mg Pyrazinamide and 275 mg ethambutol (RHZE), in addition to the three pediatric FDCs, bringing the total number of WHO recommended FDCs up to 12 (WHO, 1999). Fixed dose combination containing TH (Thiacetazone + Isoniazid), EH (Ethambutol + Isoniazid), and RH (Rifampicin + Isoniazid) are in use since last two decades.

WHO and IUATLD recommend FDCs only of proven bioavailability. The drugs given as FDCs or single preparation should be bioequivalent, which is an important criteria while replacing single preparation with FDCs.

Strategies for quality assurance of FDCs are being established by WHO and IUATLD. A simplified protocol for testing of rifampicin bioavailability has been developed, and laboratories are being identified to form an international network for quality assurance and rifampicin bioavailability testing for FDCs (Fourie, 1999b). A global mechanism for pre-qualification of FDCs has been proposed to ensure that only quality FDCs will be purchased and used (Blomberg, 1999). WHO recommended strengths of fixed dose combination formulations of essential anti-TB drugs (Table 2.3) (Laing, 2001; WHO, 2003a)
### Table 2.3 The recommended strengths of fixed-dose combination formulations of essential anti-TB drugs to be given daily.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Forms</th>
<th>Strengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHZE</td>
<td>Tablet</td>
<td>R 150 mg + H 75 mg + Z 400 mg + E 275 mg</td>
</tr>
<tr>
<td>RHZ</td>
<td>Tablet</td>
<td>R 150 mg + H 75 mg + Z 400 mg + E 275 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 60 mg + H 30 mg + Z 150 mg (pediatric)*</td>
</tr>
<tr>
<td>RH</td>
<td>Tablet</td>
<td>R 300 mg + H 150 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 150 mg + H 75 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 60 mg + H 30 mg (pediatric)*</td>
</tr>
<tr>
<td>EH</td>
<td>Tablet</td>
<td>E 150 mg + H 400 mg</td>
</tr>
<tr>
<td>TH</td>
<td>Tablet</td>
<td>T 50 mg + H 100 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T 150 mg + H 300 mg</td>
</tr>
</tbody>
</table>

*Dispersible form preferred

E = Ethambutol, H = Isoniazid, R = Rifampicin, S = Streptomycin, T = Thiacetazone, Z = Pyrazinamide

Table-2.4 and Table-2.5 show the dosage schedules for adults and children, respectively (Laing, 2001; WHO, 2003a).

### Table 2.4 Dosage schedule for FDCs of WHO-recommended strengths for adults.

<table>
<thead>
<tr>
<th>Patients's body weight (kg)</th>
<th>Initial phase:</th>
<th>Continuation phase:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 months</td>
<td>4 months</td>
</tr>
<tr>
<td></td>
<td>RHZE daily</td>
<td>RHZ daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-37</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>38-54</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5-70</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>≥71</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

R = rifampicin; H = isoniazid; Z = Pyrazinamide; E = ethambutol.
Table-2.5 WHO-recommended dosage schedule (number of tablets) for children.

<table>
<thead>
<tr>
<th>Patient’s body Weight (kg)</th>
<th>2 months</th>
<th>4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial phase:</td>
<td>Continuation phase:</td>
</tr>
<tr>
<td></td>
<td>RHZ daily</td>
<td>RH daily</td>
</tr>
<tr>
<td>≤ 7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8-9</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>10-14</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15-19</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>20-24</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>25-29</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

R = Rifampicin, H = Isoniazid; Z = Pyrazinamide.

Blomberg and co-workers has enumerated the advantages of FDCs over the traditional multiple tablet schedule (Blomberg, 2002). The following table 2.6 shows the advantage (considerable reduction in dosing frequency).

Table 2.6 Number of tablets to be taken daily in the intensive phase of TB treatment for 50 kg patient.

<table>
<thead>
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<th>Single drug tablets</th>
<th>FDC tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>No of tablets</td>
</tr>
<tr>
<td>R: 150 mg</td>
<td>3</td>
</tr>
<tr>
<td>H: 300 mg</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Z: 400 mg</td>
<td>3</td>
</tr>
<tr>
<td>E: 400 mg</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (16)</td>
</tr>
</tbody>
</table>

E = ethambutol; H = isoniazid; R = rifampicin; Z = pyrazinamide.

The risk of emergence of drug-resistant tuberculosis can be limited by adopting FDCs. However, this contention remains to be validated (WHO, 1999b). FDCs reduce the risk of...
2. Introduction

misuse of rifampicin for conditions other than tuberculosis. The transition from single-drug formulations to FDC tablets has been in process for many years, and the introduction of a four-drug FDC tablet is just one further step towards ensuring adequate treatment of tuberculosis.
2.3 Introduction to Dosage forms

In the present work, gastro-retentive dosage form of rifampicin and enteric dosage form of isoniazid were developed.

2.3.1 Gastro-retentive dosage form

Over the past three decades, the pursuit and exploration of devices designed to be retained in the upper part of the gastrointestinal (GI) tract has advanced consistently in terms of technology and diversity.

Benefits of Gastric retention:

(1) The delivery of drugs with narrow absorption window in the small intestinal region is possible.

(2) Longer residence time in the stomach for local action and action in the upper part of the small intestine, for example treatment of peptic ulcer.

(3) Improved bioavailability is expected for drugs that are absorbed readily upon release in the stomach.

(4) Beneficial for the drugs that are unstable in alkaline environment or causes irritation in the intestine.

Drawbacks of gastric retention:

(1) Aspirin and non-steroidal anti-inflammatory drugs are known to cause gastric lesions, and slow release of such drugs in the stomach is unwanted.

(2) Drugs that may irritate the stomach lining or are rapidly degraded in its acidic environment should not be formulated in gastro-retentive systems.

(3) Patient posture and dietary habit may affect the performance of formulation.

Approaches to Gastric Retention:

A variety of systems and devices such as gastro-retentive systems, raft systems, expanding systems, swelling systems, bioadhesive systems and low-density systems have been suggested (Jimenez-Castellanos, 1993, Desai, 1993; Whitehead, 1996; Iannuccelli, 1998).
Gastro-retentive systems can be based on the following:

- **Hydrodynamically balanced systems (HBS)** - Incorporated buoyant materials enable the device to float.
- **Effervescent systems** - Gas-generating materials such as carbonates are incorporated which reacts with gastric acid and produce carbon dioxide and allows system to float.
- **Low-density systems** - The formulation is fabricated with lower density than that of the gastric fluid to float.
- **High density systems** - These systems contains high density excipients which will not allow emptying from stomach.
- **Raft systems** - Carbon dioxide bubbles are entrapped in alginate gel.
- **Bioadhesive or mucoadhesive systems** - In these systems, drug is to be incorporated with bio/mucoadhesive agents to adhere to the stomach (or other GI) wall for resisting gastric emptying.
- **Swelling systems** - The materials swells in gastric fluid and does not allow the system to go out from stomach
- **Expanding systems** - These systems expands after reaching to stomach so that the size of formulations increases and does not allow emptying.

Marketed products of gastro-retentive dosage forms are shown below:

- **Valrelease®**: Floating capsule of diazepam.
- **Madopar®**: Benserazide and L-Dopa combination formulation.
- **Liquid Gaviscon®**: Floating liquid alginate preparations.
- **Topalkan®**: Aluminium magnesium antacid preparation.
- **Ahnagate Flot-Coat®**: Antacid preparation.
Table 2.7 List of drugs explored for various gastro-retentive dosage forms:

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Explored drugs (Singh, 2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microshperes</td>
<td>Aspirin, Griseofulvin, p-niroaniline, Ibuprofen, Terfenadine, Tranilast</td>
</tr>
<tr>
<td>Granules</td>
<td>Diclofenac sodium, Indomethacin, Prednisolone</td>
</tr>
<tr>
<td>Films</td>
<td>Cinnarizine</td>
</tr>
<tr>
<td>Powders</td>
<td>Several basic drugs</td>
</tr>
<tr>
<td>Capsules</td>
<td>Chlordiazepoxide HCl, Diazepam, Furosemide, L-Dopa, Benserazide, Misoprostol, Propranolol HCl, Ursodeoxycholic acid</td>
</tr>
<tr>
<td>Tablets/Pills</td>
<td>Acetaminophen, Acetylsalicylic acid, Amoxycillin trihydrate, Ampicillin, Atenolol, Chllopheniramine maleate, Cinnarizine, Diltiazem, Fluorouracil, Isosorbide dinitrate, p-Aminobenzoic acid, Piretaneide, Prednisolone, Quinidine gluconate, Riboflavine-5'-phosphate, Sotalol, Theophylline, Verpamil HCl</td>
</tr>
</tbody>
</table>
2.3.2 Introduction to enteric coated dosage form:

Enteric coated solid dosage forms are intended to pass through the stomach intact to disintegrate and release their drug content for absorption in the intestine. The design of an enteric coating may be based on the transit time required for passage to the intestines. It is accomplished through coatings of sufficient thickness. Some enteric coatings are designed to dissolve at pH ≥ 4.8 (Lachman, 1990; Lloyd, 2005).

Benefits of enteric coated dosage form:

1. Protects acid labile drugs from the gastric fluid, e.g., enzymes and certain antibiotics
2. Prevents gastric distress or nausea due to irritation of gastric wall e.g., sodium salicylate
3. Deliver drugs intended for local action in the intestines, e.g., intestinal antiseptics could be delivered to their site of action in a concentrated form and bypass systemic absorption in the stomach
4. Provides a delayed release component for repeat action tablets

Enteric coating materials may be applied to either whole compressed tablets or to drug particles or granules used in the fabrication of tablets or capsules. The coatings may be applied in multiple portions to build a thick coating or as a thin film coat. The coating system may be aqueous or organic solvent based and effective so long as the coating material resists breakdown in the gastric fluid.

An ideal enteric coating material should have the following properties:

1. Resistance to gastric fluids
2. Ready susceptibility to or permeability to intestinal fluids
3. Compatibility with most coating solution components and the drug substrates
4. Stability alone and in coating solutions.
5. Formation of a continuous (uninterrupted) film
6. Nontoxicity
7. Low cost
8. Ease of application without specialized equipment
9. Ability to be readily printed or to allow film to be applied to debossed tablets
2. Introduction

The United States Pharmacopeias (USP) disintegration test for enteric coated tablets requires that the tablets tolerate agitation in simulated gastric fluid test solution at 37 ± 2°C (no disc). After 1 hour of exposure in simulated gastric fluid, tablets should show no evidence of disintegration, cracking, or softening. Then, a disc is added to each tube, and the test is continued using simulated intestinal fluid maintained at 37 ± 2°C as the immersion fluid, for a period of time equal to two hours or to the time limit specified in the individual monograph. If all the tablets disintegrate, the product passes the test. If one or two tablets fail to disintegrate completely, the test is repeated on twelve additional tablets. To pass the disintegration test, at least sixteen out of eighteen tablets should disintegrate.

All enteric coated tablets must meet these requirements. Passing the USP enteric test does not guarantee optimal bioavailability of a particular dosage form. Several situations complicate the absorption of drug from enteric coated tablets. The pH of the stomach contents may vary from 1.5 to 4.0, with about 10% of the patients having achlorhydria. The amount of gastric fluid may vary between individuals, and for the same individual from time to time. Gastric residence time for the dosage form may range from less than half an hour to more than 4 hours depending on the time of its administration, whether it was consumed with food, and if so, the type and quantity of food. The USP disintegration test does not require a qualitative or quantitative test for the active drug after agitation in artificial gastric fluid for 1 hour. Several commercially available enteric products passed the USP enteric test, but released varying amounts of drugs in simulated gastric fluid. Most acid labile drugs need protection between pH values 1 and 5. The pH of material approaching pylorus is expected to be about 5. An ideal enteric polymer should dissolve or become permeable near and above pH 5.

A common problem associated with the retardant type of polymers (non pH dependent solubility) is failure to release the drug in the intestine. Commercial products have failed the enteric test both for lack of gastric protection and for lack of solubility in intestinal fluids. Many others passed these in vitro tests, but failed to perform adequately when studied in vivo.
List of enteric coating materials:

1. Shellac
2. Cellulose acetate phthalate (CAP)
3. Acrylate polymers (Eudragit soluble at pH greater than 5.)
4. Hydroxy propyl methyl cellulose phthalate (HPMCP)
5. Polyvinylacetate phthalate
6. Diethyl phthalate
2. Introduction to drugs

2.4 Introduction to rifampicin (RIF)

Structure:

Physicochemical Characteristics:

<table>
<thead>
<tr>
<th>Characters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C_{43}H_{58}N_{4}O_{12}</td>
</tr>
<tr>
<td>Morphology</td>
<td>Odorless, brick-red to reddish-brown crystalline powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>185°C</td>
</tr>
<tr>
<td>pH</td>
<td>4.5 - 6.5 (1% w/v suspension of rifampicin)</td>
</tr>
<tr>
<td>pKa</td>
<td>pKa = 1.7 (naphthalene hydroxyl groups), pKa = 7.9 (piperazine nitrogen)</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>Log P = 1.2 (octanol / pH 7.2 aqueous buffer)</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>823</td>
</tr>
<tr>
<td>Forms</td>
<td>Six different forms such as monohydrate, dihydrate, two amorphous form, 1:1 rifampicin:acetone solvate and 1:2 rifampicin:2-pyrrolidone olvate (Henwood, 2001).</td>
</tr>
<tr>
<td>Solubility</td>
<td>Slightly soluble in water, ethanol, acetone, carbon</td>
</tr>
</tbody>
</table>
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tetrachloride and ether; practically insoluble in butanol, cyclohexan, glycerol and propylene glycol; soluble in ethyl acetate, methanol and tetrahydrofuran; and freely soluble in chloroform and dimethyl sulfoxide.

<table>
<thead>
<tr>
<th>Effect of pH on Solubility</th>
<th>Highly soluble at pH 1-2 (1 in 5 of 0.1M HCl), less soluble at pH 7.4 (1 in 100 of phosphate buffer) (Gallo, 1976); soluble in approximately 10, 250 and 350 at pH 2, 5.3 and 7.5, respectively (Singh, 2000).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability</td>
<td>Stable for at least 5 years at 25°C in solid state; in acidic medium (pH 2.3), it is hydrolyzed to 3-formyl rifampicin sv (prone to precipitate) and 1-amino-4 methylpiprazine (Gallo, 1976); in basic media (pH 8.0) and in the presence of atmospheric oxygen rifampicin is oxidized to rifampicin quinine which can be prevented by addition of ascorbic acid (Gallo, 1976); decomposition is slower in neutral media (Gallo, 1976); at pH 8.2, various 25-desacetyl derivatives are formed (Singh, 2000).</td>
</tr>
</tbody>
</table>

**Pharmacology and mechanism of action**

Rifampicin has unique ability to kill semi dormant tubercle bacilli when they undergo sporadic burst of metabolism and growth (Ellard, 1999). It is the only drug which has this property and therefore, is the mainstay of tuberculosis therapy. Rifampicin has a broad spectrum antibacterial activity and acts by inhibiting DNA dependent RNA polymerase (DDRP), inhibiting transcription. It specifically inhibits the transition from synthesis of short oligonucleotides to full length transcripts. It had been suggested that the naphthalene ring of rifampicin bounds to an aromatic amino acid ring in the DDRP protein. DDRP is metalloenzyme that contains two zinc atoms. Oxygen at C-1 and C-8 of rifampicin chelate a zinc atom that increases binding of rifampicin to DDRP and finally, the oxygen at C-21 and C-23 form strong hydrogen bonds to the DDRP. The binding of...
rifampicin to DDRP results in the inhibition of RNA-synthesis. Resistance can be developed rapidly in a one step process and is thought to be due to chemical modification of microbial DNA dependent RNA polymerase, resulting from a chromosomal mutation. It has a minimum inhibitory concentration of 0.005-0.02 μg/ml against Mycobacterium tuberculosis (Mandell, 1980; Rang, 1995; Sensi, 1996).

Use and administration
The usual oral dose of rifampicin in tuberculosis is 8 to 12 mg per kg body weight given daily before food, patients weighing less than 50 kg body weight are often given 450 mg daily while patients more than 50 kg in weight receive 600 mg. Children may be given 10 to 20 mg per kg to a maximum daily dose of 600 mg. In patients with impaired liver function, the dose should generally not exceed 8 mg per kg daily however, it has been reported that in the patients with chronic renal failure, it is not necessary to reduce dosage of rifampicin (Rang, 1995). Intermittent schedules are often employed although these may increase incidence of side effects, depending on the schedules adopted; dosage of up to 900 mg have been given twice weekly (Mandell, 1980; Rang, 1995; Sensi, 1996).

Adverse effects
Intermittent use of rifampicin may increase the chance of patient developing the flue like syndrome (chills, difficult breathing, dizziness, fever, headache, muscle and bone pain, shivering) as well as acute haemolysis or renal failure. It can produce hypersensitivity reactions characterized by itching, redness and rashes on the skin. Some adverse events like GI disturbance, diarrhea and stomach cramp may need medical attention; while other mild adverse reactions include reddish to orange discoloration of urine, feces, saliva, sputum sweat and tears (Mandell, 1980; Rang, 1995; Sensi, 1996).

Pharmacokinetics
Absorption
Rifampicin is well absorbed from the gastrointestinal tract, with peak plasma levels achieved within 1 to 4 hours after oral administration, although food may delay absorption (Verbist 1968; Keberle, 1971, Sielgeler, 1974). Also, gastric pH is of importance and acidification of gastric juice increases absorption and serum
2. Introduction

concentrations. Singh and co-workers reported that rifampicin is well absorbed from stomach due to its high solubility at pH 1-2 (Singh, 2003).

Distribution

Rifampicin readily diffuses into most organs, tissues, bone and body fluids, including exudates into tuberculous lung cavities (Acocella, 1967). Therapeutic concentrations are achieved in saliva reaching 20% of serum concentrations. High concentrations appear in the lachrymal glands and tears. The urine is colored orange to brick-red. The volume of distribution is approximately 1L/kg. Serum binding has been estimated at 60-80% (Acocella, 1971) with approximately 30 % with the serum albumin fraction where it may compete, for instance, with warfarin anticoagulants. It is reported that rifampicin is highly protein bound to an extent of 84-91% (Jack 1992; USP DI, 1996). 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 (Jack 1992; USP DI, 1996). 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 (Nahata, 1990). There is evidence that rifampicin can cross the placenta.

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 with increased capacity for biliary excretion (Devam, 1985). Depending on the dose of rifampicin, one third to one eighth may be excreted in the bile. Singh and co-workers reported that rifampicin is well absorbed from stomach due to its high solubility at pH 1-2 or as unchanged rifampicin (Singh, 2003). The unchanged rifampicin is reabsorbed, creating an enterohepatic circulation, whereas the 25-desacetyl rifampicin is poorly absorbed (Teuinissen, 1984). Other identified metabolites include rifampicin-quinone, desacetyl rifampicin quinone and 3-formyl rifampicin sv (Reynolds, 1993). A potent enzyme inducer, rifampicin, stimulates its own
metabolism in liver and the biliary excretion of desacetylrifampicin (Doulas, 1999). On repeated administration of rifampicin, its plasma levels are found to decrease.

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 (Keberle, 1971), but repeated administration induces hepatic endoplasmic reticular enzymes, including deacetylation with reduction in serum half-life and area under curve (AUC); typical figures are given in Table-2.5.2.

Table 2.9 Reduction in serum half-life and area under curve.

<table>
<thead>
<tr>
<th>Days</th>
<th>Half-life (h)</th>
<th>AUC(0-12 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>3.4</td>
<td>131.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>2.9</td>
<td>84.6</td>
</tr>
<tr>
<td>Day 6</td>
<td>2.5</td>
<td>100.5</td>
</tr>
<tr>
<td>Day 14</td>
<td>2.1</td>
<td>87.7</td>
</tr>
</tbody>
</table>

Excretion

Rifampicin is mainly eliminated in bile gets reabsorbed and undergoes enterohepatic circulation. Although, the kidney is not the main excretion pathway for rifampicin or its metabolites, urinary concentrations increase with doses above 450 mg when the biliary excretion pathway is more saturated. In infants, here hepatic mechanisms are less fully developed, more rifampicin is excreted in the urine and there is less change with time because enzyme induction is low at this age. Amount excreted in urine increases with increasing doses and up to 30% of dose of 900 mg may be excreted in urine, about half of it within 24 h (Brufani, 1964; Acocella, 1978; Jack, 1992; Reynolds, 1993; Sensi, 1996; USP DI, 1996; Doulas, 1999). About 40% is excreted in bile. About 60-65% dose appears in feces. Within 24 h, 30-30% of unchanged drug and active metabolite get excreted in urine (600 mg single dose oral administration). About 6-15% of dose is excreted as unchanged drug and 15% of dose appears as active metabolite (25-desacetyl rifampicin) in urine. Seven % of dose is excreted as inactive 3-formyl rifampicin sv (USP DI, 1996).
2.4.2 Introduction to isoniazid (INH)

Structure:

\[
\text{CONHNH}_2
\]

Physiochemical characteristics:

**Table 2.10 Physicochemical characteristics of isoniazid.**

<table>
<thead>
<tr>
<th>Characters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>( \text{C}_6\text{H}_7\text{N}_2\text{O} )</td>
</tr>
<tr>
<td>Morphology</td>
<td>odorless, colorless or white crystalline powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>171-174°C</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>137.14</td>
</tr>
<tr>
<td>pH</td>
<td>5.5-6.5 (1% w/v solution of INH)</td>
</tr>
<tr>
<td>pKa</td>
<td>( \text{pKa} = 1.8 ) (hydrazine nitrogen), ( \text{pKa} = 3.5 ) (pyridine nitrogen), ( \text{pKa} = 10.8 ) (acidic group)</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>Log ( P = 1.1 ) (octanol/pH 7.4 aqueous buffer)</td>
</tr>
<tr>
<td>Solubility</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>Solubility</td>
</tr>
<tr>
<td>Water</td>
<td>2.5 g/100ml</td>
</tr>
<tr>
<td>Ethanol (25°C)</td>
<td>2 g/100ml</td>
</tr>
<tr>
<td>Ethanol (boiling)</td>
<td>10 g/100ml</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.1g/100ml</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>very slightly soluble</td>
</tr>
<tr>
<td>Benzene</td>
<td>insoluble</td>
</tr>
<tr>
<td>Stability</td>
<td>The aqueous solution is stable at room temperature for about 45 days. The stability is better when stored at 4°C. Isoniazid degrades to isonicotinic acid, isonicotinamide and 1, 2-</td>
</tr>
</tbody>
</table>
Pharmacological and mechanism of action:
Isoniazid is bactericidal in vitro and in vivo against actively dividing tubercular bacilli and less active against non-replicating bacilli. It primarily inhibits the synthesis of long chain mycolic acids which are the unique constituents of mycobacterial cell wall. In low concentrations, it may prevent elongation of very long chain fatty acid precursors of mycolic acids. It mainly targets InhA protein which catalyzes the nicotinamide adenine dinucleotide (NADH) – specific reduction of 2-trans-enoyl-acyl carrier protein, an essential step in fatty acid elongation. Resistance to isoniazid by mycobacterium tuberculosis can be mediated by substitution of alanine for serine-94, which perturbs the hydrogen bonding network that stabilizes NADH-binding to the protein (Dessen, 1995). On the other hand, it has been proposed that INH penetrates mycobacterial cell wall with the help of hydrazide group acting as carrier. Isoniazid is then oxidized enzymatically to isonicotinic acid, which is ionized at intracellular pH and hence, it cannot return across the membrane. The accumulated isonicotinic acid in the cell gets quaternized and competes with nicotinic acid in the formation of NAD. This analog of NAD, containing isonicotinic acid, can no longer function as the natural co-enzymes in metabolic functions of the bacterial cell (Lemke, 1995).

Dose and administration:
Isoniazid is usually administered by oral route but it may also be given intramuscularly when oral therapy is not possible. The usual dose of isoniazid for adults is 300 mg or 5 to 8 mg/kg/day. Severely ill patients may be given a dose as high as 10 mg/kg/day. Children tolerate larger doses of isoniazid than do adults and may be given 10 to 20 mg/kg/day. If the drug is given to adults twice weekly then the dosage is usually 600-900 mg.
minimum inhibitory concentration of isoniazid for mycobacterium tuberculosis is 0.05-0.25 mg/l (Krishnamurti, 1975)

Uses:
It is used in the treatment of pulmonary and extra-pulmonary tuberculosis and also as a prophylactic in tuberculosis.

Adverse effects:
It has been reported that isoniazid has relatively low toxicity and only about 5% of patients may experience some form of adverse effect during treatment if pyridomine is administered concurrently. Hypersensitivity reactions include various skin eruption, fever, lymphademopathy, hepatotoxicity, hematological changes, arthritic symptoms and vasculitis. Peripheral neuropathy may be common if pyridoxine is not administered concurrently. Isoniazid induced pyridoxine deficiency causes pellagra. Isoniazid is reported to undergo interactions with alcohol, antacids and anticoagulants (USPDI, 1996b).

Pharmacokinetics:

Absorption
Isoniazid is absorbed rapidly and completely after oral administration primarily from small intestine. Peak plasma concentrations of 3-7 mg/l is achieved 1 to 2 hour after oral administration of normal therapeutic doses to adults (Hurwitz, 1974). Absorption and bioavailability of isoniazid is reduced when it is administered with high-carbohydrate meals and various antacids (Holdiness, 1984). It undergoes appreciable presystemic metabolism in the wall of small intestine and liver. There is no measurable difference in the peak isoniazid concentration in rapid and slow acetylators after intravenous administration.

Distribution
Isoniazid shows negligible protein binding (0-10%). It is distributed in different parts of the body (volume of distribution, 0.6-0.8 l/kg). A significant amount of concentration of the drug is found in CSF, saliva, lung, skin, and feces. It crosses placental barrier. However, a survey has concluded that the use of isoniazid is not contraindicated during pregnancy, since the risk to fetus is minimal (Nitya, 1995).

Metabolism
Isoniazid undergoes extensive metabolism, the extent of which is largely determined by acetylator phenotype. It metabolizes in the mucosal cells of the small intestine and the liver into N-acetylisoniazid. Other metabolite includes isonicotinic acid, monoacetylhydrazine and isoacetylhydrazine. All the metabolites of isoniazid are devoid of antitubercular activity and are less toxic (Nitya, 1995).

Elimination
Elimination of the isoniazid is dependent upon its genetically controlled rate of acetylation. In a patient with normal renal function, about 50-70% of dose appears in the urine in 24 hour, partly as unchanged isoniazid but mainly in the form of its metabolite. Of this amount, 93% of isoniazid is excreted in urine in fast acetylators in form of N-acetylisoniazid and 63% in slow acetylators as N-acetylisoniazid (USP DI, 1996b). The plasma half-life of isoniazid has been reported to be 0.5 to 1.5 hour in rapid acetylators and 2 to 6.5 hour in slow acetylators. The values of elimination half-life of isoniazid in rapid acetylators ranges from about 45 min to 110 min, where as in slow acetylators the values ranges from 110 min to more than 400 min. Rapid acetylators excrete small amounts of the drug unchanged in urine (about 3% of dose) whereas slow acetylators may excrete up to 30% of dose as unmetabolized isoniazid (Gibaldi, 1984). After ingestion of an oral dose of isoniazid the urine contains isoniazid, pyruvic acid hydrazone, α-ketoglutaric acid hydrazone, acetylisoniazid, isonicotinic acid, isonicotinyl glycine, monoacetylhydrazine and diacetylhydrazine.
2.5 Introduction to Excipients

2.5.1 Introduction to HPMC K4 M

Hypromellose is an odorless, and tasteless, white or creamy white fibrous or granular powder. Hypromellose is widely used in oral and topical pharmaceutical formulations. In oral products, hypromellose is primarily used as a tablet binder, in film coating, and as an extended release tablet matrix. Concentrations between 2% and 5% w/w may be used as a binder in either wet or dry-granulation processes. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10-80% w/w in tablets and capsules. Depending upon the viscosity grade, concentrations of 2-20% w/w are used for film forming solutions to film coat tablets. Lower viscosity grades are used in aqueous film coating solutions, while higher viscosity grades are used with organic solvents (Rowe, 2003a)

Hypromellose is also used as a suspending and thickening agent in topical formulations, particularly ophthalmic preparations. Compared with methylcellulose, hypromellose produces solutions of greater clarity, with fewer undispersed fibers present, and is therefore preferred in formulations for ophthalmic use. Hypromellose at concentrations between 0.45-1.0% w/w may be added as a thickening agent to vehicles for eye drops and artificial tear solutions (Rowe, 2003a)

Hypromellose is also used as an emulsifier, suspending agent, and stabilizing agent in topical gels and ointments. As a protective colloid, it can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments (Rowe, 2003a)

In addition, hypromellose is used in the manufacture of capsules, as an adhesive in plastic bandages, and as a wetting agent for hard contact lenses. It is also widely used in cosmetics and food products (Rowe, 2003a)

Typical Properties:

Acidity/Alkalinity: $\text{pH}= 5.5-8.0$ for a 1% w/w aqueous solution

Ash: 1.5-3.0%, depending upon the grade.

Autoignition temperature: 360° C

Density (Bulk): 0.341 g/cm$^3$
2. Introduction

Density (tapped): 0.557 g/cm³
Density (true): 1.326 g/cm³

Melting point: Browns at 190-200° C; chars at 225-230° C. Glass transition temperature is 170-180° C.

Moisture content.

Hypromellose absorbs moisture from the atmosphere, the amount of water absorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Solubility:
It is soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, mixtures of water and alcohol. Certain grades of hypromellose are soluble in aqueous acetone and other organic solvents (Rowe, 2003a)


Dynamic viscosity:
Aqueous solutions are most commonly prepared, although hypromellose may also be dissolved in aqueous alcohols such as ethanol and propan-2-ol provided the alcohol content is less than 50% w/w. Dichloromethane and ethanol mixtures may also be used to prepare viscous hypromellose solutions. Solutions prepared using organic solvents tend to be more viscous; increasing concentration also produces more viscous solutions. Nominal viscosity values for 2% (w/v) aqueous solutions of HPMC K4 M is equal to 4000 mPas at 20° C and range of viscosity is equal to 3000-5600 mPas.

To prepare an aqueous solution, it is recommended that hypromellose is dispersed and thoroughly hydrated in bout 20 to 30% of the required amount of water. The water should be vigorously stirred and heated to 80-90° C, and then the remaining hypromellose is added. Cold water should then be added to produce the required volume. When a water miscible organic solvent such as ethanol, glycol, or mixtures of ethanol and dichloromethane is used, the hypromellose should first be dispersed into an organic
solvent, at a ratio of 5-8 parts of solvent to 1 part of hypromellose, cold water is then added to produce the required volume (Rowe, 2003a).

2.5.2 Introduction to Polyethylene Oxide (PEO)

Polyethylene oxide is white to off-white, free-flowing powder. Polyethylene oxide can be used as a tablet binder at concentrations of 5-85%. The higher molecular weight grades provide delayed drug release via the hydrophilic matrix approach. The relationship between swelling capacity and molecular weight is a good guide when selecting products for use in immediate or sustained release matrix formulations. Polyethylene oxide has been shown to be an excellent mucoadhesive polymer. Low levels of polyethylene oxide are effective thickeners, although alcohol is usually added to water-based formulations to provide improved viscosity stability. Polyethylene oxide films demonstrate good lubricity when wet. This property has been utilized in the development of coatings for medical devices. Polyethylene oxide can be radiation crosslinked in solution to produce a hydrogel that can be used in wound care applications (Rowe, 2003b).

Typical properties

- **Angle of repose:** 34°
- **True density:** 1.3gm/cc
- **Melting point:** 65-70°C
- **Moisture content:** <1%
- **Solubility:** Polyethylene oxide is soluble in water and a number of common organic solvents such as acetonitrile, chloroform, and methylene chloride. It is insoluble in aliphatic hydrocarbons, ethylene glycol, and most alcohols (Rowe, 2003b).

2.5.3 Introduction to Eudragit L100-55

It is prepared by spray-drying Eudragit L 30 D-55. It is white free-flowing powder that is redispersible in water to form latex that has properties similar to those of Eudragit L 30 D-55. It is used as an enteric coating film former for solid-dosage forms. The coatin is
resistant to gastric juice but dissolves readily at above pH 5.5. It is commercially available as a redispersible powder (Rowe, 2003c).

**Typical Properties:**

*Acid value:* 300-330  
*Bulk density:* 0.390 gm/cc  
*Tapped density:* 0.424 gm/cc  
*True density:* 0.821-841 gm/cc  
*Refractive index:* 1.387-1.392  
*Solubility:* Soluble in acetone, alcohols, solution with pH more than 5.5  
*Viscosity:* 100-200 mPas

### 2.5.4 Introduction to Hydroxypropyl methylcellulose phthalate

Hypromellose phthalate occurs as white to slightly off-white, free-flowing flakes or as a granular powder. It is odorless or with a slightly acidic odor and has a barely detectable taste. Hypromellose phthalate is widely used in oral pharmaceutical formulations as an enteric coating material for tablets of granules. Hypromellose phthalate is insoluble in gastric fluid but will swell and dissolve rapidly in the upper intestine. Generally, concentrations of 5-10% of hypromellose phthalate are employed with the material being dissolved in either a dichloromethane: ethanol (50:50) or an ethanol: water (80:20) solvent mixture. Hypromellose phthalate can normally be applied to tablets and granules without the addition of a plasticizer or other film formers, using established coating techniques. However, the addition of a small amount of plasticizer or water can avoid film cracking problems; many plasticizer or water can avoid film cracking problems; many commonly used plasticizers, such as diacetin, triacetin, diethyl and dibutyl phthalate, castor oil, acetyl monoglyceride, and polyethylene glycols are compatible with hypromellose phthalate. Tablets coated with hypromellose phthalate disintegrate more rapidly than tablets coated with cellulose acetate phthalate (Rowe, 2003d).

Hypromellose phthalate can be applied to tablet surfaces using a dispersion of the micronized hypromellose phthalate powder in an aqueous dispersion of a suitable
plasticizer such as triacetin, triethyl citrate, or diethyl tartrate along with a wetting agent. Hypromellose phthalate may be used alone or in combination with other soluble or insoluble binder in the preparation of granules with sustained drug release properties the release and insoluble in saliva, it can also be used as a coating to mask the unpleasant taste of some tablet formulations (Rowe, 2003d).

Typical properties:

**Angle of repose:** 37° for HP 50
39° for HP 55
38° for HP 55S

**Density:** 1.82gm/cc for HP 50
1.65gm/cc for HP 55

**Bulk density:** 0.278gm/cc for HP 50
0.275gm/cc for HP 55
0.239 gm/cc for HP 55S

**Tapped density:** 0.343gm/cc for HP 50
0.306 gm/cc for HP 55
0.288 gm/cc for HP 55S

**Melting point:** 150°C.

**Moisture content:**
Hypromellose phthalate is hygroscopic; it takes up 2-5% of moisture at ambient temperature and humidity conditions.

**Solubility:**
Readily soluble in a mixture of acetone and methyl or ethyl alcohol (1:1), in a mixture of methyl alcohol and dichloromethane (1:1), and in aqueous alkali, practically insoluble in water and dehydrated alcohol and very slightly soluble in acetone (Rowe, 2003d).

### 2.5.5 Introduction to Sodium Alginate

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrant; it has
been used as a diluent in capsule formulations. Sodium alginate has also been used in the preparation of sustained release oral formulations since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions (Rowe, 2003e).

In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams and gels, and as a stabilizing agent for oil-in-water emulsions (Rowe, 2003e).

Recently, sodium alginate has been used for the aqueous Microencapsulation of drugs, in contrast with the more conventional Microencapsulation techniques which use organic solvent systems. It has also been used in the formation of nanoparticles (Rowe, 2003e). The adhesiveness of hydrogels prepared from sodium alginate has been investigated and drug release from oral mucosal adhesive tablets based on sodium alginate has been reported. Other novel delivery systems containing sodium alginate include an ophthalmic solution that forms a gel in situ when administered to the eye and a freeze dried device intended for the delivery of bone growth factors. Hydrogel systems containing alginates have also been investigated for delivery of proteins and peptides (Rowe, 2003e).

Therapeutically, sodium alginate has been used in combination with an H2 receptor antagonist in the management of gastroesophageal reflux and as a haemostatic agent in surgical dressings. Alginate dressings, used to treat exuding wounds, often contain significant amounts of sodium alginate as this improves the gelling properties (Rowe, 2003e).

**Typical properties:**

**Acidity/Alkalinity:** pH \( \approx 7.2 \) for a 1%w/v aqueous solution.

**Solubility:**

Practically insoluble in ethanol, ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3. It is slowly soluble in water, forming a viscous colloidal solution (Rowe, 2003e).

**Viscosity:**

Various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at 20°C, will have a
 viscosity of 20-4000 mPas (20-400cP). Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ion. Above pH 10, viscosity decreases (Rowe, 2003e).
2.6 Introduction to Methods

Tablets are prepared by drug granulation, wet granulation or direct compression. Direct compression is feasible for a limited number of substances due to problems such as poor powder flow properties, low tablet strength, capping and segregation (Remon, 2001a). Granulation is designed to overcome these problems and usually results in better flowability and compactibility of the powder. There are two granulation methods (Banker, 1990).

2.6.1 Granulation methods

(a) Wet granulation

It involves addition of a suitable binder solution or a granulating agent to the blend of active ingredient/s and adjuvants to prepare damp mass. Subsequently, screening and drying of granules are carried out. The dried granules are resifted to break the large agglomerates. The dried granules are finally mixed with adjuvants like extragranular disintegrating agent, lubricant and glidant. The granules ready for compression are tableted using a tablet press. Wet granulation is probably the preferred process for the production of colored tablets and tablets containing potent drugs with minimum tablet-to-tablet variation. Wet granulation includes high shear granulation, fluid bed granulation, extrusion granulation, continuous granulation and all in one granulation (Shangraw, 1988; Shangraw, 1989).

Benefits:

(1) Improves flow by increasing particle size and sphericity.

(2) Optimum fill density can be achieved by adjusting the process to create the optimum particle size distribution.

(3) Compressibility and consolidation can be improved via the choice of the correct binder and the adjustment of moisture content of the granules.

(4) Reduces air entrapment, level of dust formation and cross contamination

(5) Dissolution can be modified through adjuvant selection (hydrophilization to improve wetting or to obtain a modified release inclusion of matrixing agent).
2. Introduction

Drawbacks:
(1) The wet granulation process involves the use of many unit operations. Each unit operation gives rise to its own specific complications. More the number of unit operations, more validation and documentation are required.

(2) Wet granulation is time consuming (particularly drying step) and labour intensive process compared to direct compression.

(3) Due to more numbers of processing steps, a large number of processing equipments and vessels are required that may give rise to cross contamination and/or material loss due to adhesion of mass to vessels.

(4) Due to above enlisted limitations, the final manufacturing cost of tablet is high compared to direct compression.

(5) Many drugs are sensitive to heat and/or moisture. The wet massing and drying step can alter the stability of such drugs.

(6) On storage, change in the disintegration time or the dissolution rate may occur.

Apart from the above mentioned drawbacks, the wet granulation requires critical monitoring of the following points:

(1) The type, concentration, viscosity, rate of addition, distribution and massing time of binder solution.

(2) The effect of temperature, time, initial moisture level, rate drying on stability of active pharmaceutical ingredient.

(3) Wet and dry sieving can affect the nature of the granules formed as well as the particle size distribution of the granules due to overfeeding. Final mixing, by its grinding action, can also change the particle size distribution, thus affecting the compaction characteristics of the granules.

(4) Difficulties in analysis may be observed for low dosage drugs due to incomplete extraction, if the active ingredient is complexed by the binder, or adsorbed on to one of the formula ingredient.
2. Introduction

(b) Roller compaction

Roller compaction is also referred to as dry granulation, slugging or double compression. The admixture of active ingredient/s and excipients is formed into large tablets or slugs in a roller compactor (chilsonator). These slugs are milled into granules of predetermined size. The milled slugs are compressed into tablets after mixing with auxiliary adjuvants such as disintegrant, lubricant and glidant (Banker, 1990).

Benefits:

1. Permits mechanical handling without loss in mix quality
2. Eliminates the problems due to heat and moisture
3. Improves flow of powders due to increase in particle size
4. Increases the final compressibility by decreasing the elastic recovery

Drawbacks:

1. High amount of reprocessing due to poor yield.
2. Chances of particle erosion and segregation during mixing and handling.
3. Limitation in mixing of colour variety
4. Expensive because it requires specialized equipment and more power consumption.

2.6.2 Cold Extrusion Technique

In some cases, however, problems still exist during the large scale production of tablets. There is also an increasing interest for continuous operation in the pharmaceutical industry. It is clear that a single step continuous granulation/tabletting process could provide advantages, such as reduced investment, reduced labour cost and easier automation of the process (Remon, 2004).

Several researchers have successfully used the hot melt extrusion technique for the continuous production of sustained release tablets (Prapairakul, 1991; Griinhagen, 1994;
2. Introduction

Sprockel, 1997; Zhang, 2000), The potential of cold extrusion as a continuous granulation technique has been less intensely examined (Gamlen, 1986; Lindberg, 1988; Kleinebudde, 1993). Remon and co-workers reported the granulation of α-lactose monohydrate using cold extrusion (Remon, 2001). They indicated that a twin screw extruder equipped with a proper die plate (e.g. having a aperture of 9 mm in diameter) could be suitable for the production of compact extrudates. They further investigated cold extrusion technique for the continuous production of tablets containing components with poor flow and compression properties (Remon, 2001a).

In this method, wet mass is prepared using the powders of drugs, excipients and binders. This wet mass is extruded from twin screw extruder with the fixed diameter die plate. The extruded mass is cut to suitable size and then oven dried for specific time and temperature. After drying, tablets are evaluated for quality.

Benefits:

(1) The tablets are porous as compared to that produced on rotary tablet machine. It is therefore suitable for tablet production of formulations with poor disintegration properties.

(2) The cold extrusion could be advantageous method for tabletting/granulating, considering ease of reproducibility.

Drawbacks:

(1) Water content may affect the tabletting process which shows influence on the tablet properties.

(2) Process parameter should be maintained throughout production of the batches.

2.6.3 Pan Coating Method

The application of materials on to the surface of compressed tablets has been practiced for 150 years. The reasons for doing this are to hide an objectionable taste or odor, to protect an unstable core ingredient, to impart an aesthetic appearance, or to separate incompatible ingredients by including one in the core tablet and the other in the coating. With time, the pan coating of tablets has changed from the art of earlier years in to science due to introduction of accela-cota machine. It is no longer entirely dependent
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upon the skill of the tablet coater. The design of new equipment, the development of new coating materials, advances in technology, and recognition of the importance for rapid release of medicaments from the core tablet have all contributed to improved products (Stuart, 1980).

2.6.4 Modified Emulsification Method

Many researchers reported the preparation of alginate microspheres by ionotropic gelation and cross-linking with calcium salts and polyions such as polylysine, chitosan, etc. (Hari, 1996a, b; Gonzalez Ferreiro, 2002; Gonzalez-Rodriguez, 2002; Lucinda-silva and Evangelista, 2003). Ain reported the preparation of alginate microspheres of isoniazid by syringe extrusion method. However, due to the higher aqueous solubility of isoniazid, low entrapment efficiency was observed (Ain, 2003). Rastogi and co-workers reported a modified emulsification method of alginate microspheres of isoniazid for oral sustained drug delivery with improved entrapment efficiency. (Rastogi., 2007).

The emulsification method was utilized for the preparation of microspheres followed by cross-linking with calcium chloride (Wan, 1989; Poncelet, 1992, 1999; Fundueanu, 1998; Heng, 2003). The drug was dispersed in aqueous solution of sodium alginate. The aqueous phase was emulsified in light liquid paraffin in the ratio 1:10 containing surfactant using a mechanical stirrer. The solution of calcium chloride dissolved in mixture of methanol and isopropyl alcohol was slowly added to the emulsion and stirred to assure efficient cross-linking. Microspheres were collected by filtration, washed with isopropyl alcohol thrice and finally air dried at room temperature. Variables like polymer concentration, drug-polymer ratio, concentration of cross-linking agent and time required for cross-linking were considered in the optimization of the formulation. In the present study, the enteric microcapsules of isoniazid were fabricated using modified emulsification method and effect of concentration of polymers in drug release behavior was examined.
Introduction to analytical method for determination of rifampicin and isoniazid

2.7.1 Dual wavelength spectrophotometric method for analysis of rifampicin and its degradation product 3-formyl rifampicin SV

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of a component of interest in a mixture containing an interfering component with close absorption maxima ($\lambda_{\text{max}}$).

For elimination of an interferent component, two specific wavelengths are chosen:

- The first wavelength $\lambda_1$ at which maximum absorbance is observed for the pure component of interest and;
- Second wavelength $\lambda_2$, is the wavelength at which the absorbance of the interfering component is equal to the absorbance of the interfering component at $\lambda_1$.

In the proposed procedure (Shishoo, 1999), the absorbance of rifampicin alone in a mixture of rifampicin and 3-formyl rifampicin SV was determined using dual wavelength data processing program to remove the interference of 3-formyl rifampicin SV to the absorbance at 475 nm ($\lambda_1$), the wavelength of maximum absorbance for rifampicin. Another wavelength was found out at which the absorbance of 3-formyl rifampicin SV was equal to its absorbance at 475 nm. This was confirmed by measuring the absorbance of various dilutions of 3-formyl rifampicin SV in chloroform at 475 nm and 507 nm, respectively. The absorbance at these two wavelengths was found to be equal. These two selected wavelengths were employed to determine the concentration of rifampicin from the mixture of rifampicin and 3-formyl rifampicin SV. The difference in absorbance at these two wavelengths ($A_{475-507}$) cancels out the contribution of absorbance of 3-formyl rifampicin SV in measurement of rifampicin at 475 nm and the difference in absorbance is proportional to the concentration of rifampicin in the mixture.
This was also confirmed by determining the absorbance of mixtures of various concentrations of rifampicin, keeping 3-formyl rifampicin SV concentration constant, at these wavelengths. It was observed that with the increase in rifampicin concentration, there was corresponding increase in difference of the absorbance values, $\Delta A$ ($A_{475} - A_{507}$). It was also found that this difference in absorbance values was linear in the range of 5-50 $\mu$g/ml of rifampicin with correlation coefficient ($r$) equal to 0.998 in presence of 3-formyl rifampicin SV.

Further, the difference in the absorbance values at 475 nm and 507 nm, $\Delta A$, for rifampicin alone also showed linear relationship between range of 5 to 50 $\mu$g/ml of rifampicin. These results confirm the suitability of proposed method to determine rifampicin in the presence of 3-formyl rifampicin SV. Similarly, 3-formylrifampicin SV was determine in presence of rifampicin using the difference of wavelengths, $\Delta A = A_{492} - A_{457}$.

2.7.1 Spectrophotometric method for analysis of Isoniazid

Absorbance of isoniazid solution was measured at $\lambda_{\text{max}}$ equal to 265 nm (IP, 1996).
2.8 References


Acocella, G., Pagani, V., Marchetti, M., Baroni, C.G. and Nicolis, F. B.,


Banker, G.S., Anderson, N.R., Tablets in: The Theory and Practice of Industrial
Pharmacy. Eds. Lachman, L., Lieberman, H. A., Kanig, J. L., Philadelphia USA,


Dessen, A., Quemard, A., Blanchard, J. S., Jacob, W. R. and Jr, Sachettini, J. C.,
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Indian pharmacopoeia, isoniazid tablets, 409, 1996.


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