Chapter One

Introduction
Chapter 1

1. INTRODUCTION

Tuberculosis is a chronic granulomatous disease and a major health problem in developing countries. About 1/3rd of the world population is infected with Mycobacterium tuberculosis. It is apprehended that unless urgent action is taken, >15 million people worldwide, including >4 million in India will die from tuberculosis (TB) in the first decade of 21st century (WHO, 2000). As per ICMR (2001) estimates, >40% adult in India are infected with TB, nearly 2 million people develop active disease every year and about 0.5 million people die from it.

A new dimension has got added in the 1980s due to the spread of HIV with high prevalence of tuberculosis and Mycobacterium Avium Complex (MAC) infection among these patients. HIV is estimated to have infected 3.5 million people in India (WHO, 1999) and these patients are especially vulnerable to severe form of tubercular/MAC infection. While lately, the increase in TB cases associated with HIV infections have been halted in the U.S.A., no such trend is apparent in India. Emergence of ‘multi drug resistant’ (MDR) TB with a reported overall incidence of 13% in 35 countries is threatening the whole future of current antitubercular chemotherapy. WHO in March, 1993 declared tuberculosis as a ‘Global health emergency’ (Anon, 1997). Remarkable progress has been made in the last 50 years since the introduction of streptomycin in 1947 for the treatment of TB. Its full therapeutic potential could be utilized only after the introduction of isoniazid in 1952.

The discovery of ethambutol in 1961, rifampicin in 1962 and redefinition of the role of pyrazinamide has changed the strategies in the chemotherapy of TB. Since 1970 the efficacy of short course (6-9 months) and domiciliary regimens has been demonstrated.

Clinically TB is of two types- pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB). Pulmonary tuberculosis is the commonest form of TB. In extrapulmonary tuberculosis highly vascular areas such as lymph nodes, meninges, kidney, spine and growing ends of the bones are commonly affected. The other site that may affect includes pleura, pericardium, peritoneum, liver, GIT, genitourinary tract (GUT) and skin. According to the international classification EPTB is defined as tuberculosis of organs other than the lungs, such as pleura, lymph nodes, abdomen, genitourinary tract, skin, joints, bone, tubercular meningitis, tuberculoma of the brain etc. (CTD, 1997).

Classification of EPTB - EPTB is classified as seriously ill and not seriously ill types which are dependent on the site of the disease (Table1.1).
Table 1.1. RNTCP* classification of EPTB

<table>
<thead>
<tr>
<th>Serious cases</th>
<th>Non-serious cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>➤ Disseminated tuberculosis</td>
<td>➤ Unilateral tuberculosis</td>
</tr>
<tr>
<td>➤ Pericarditis tuberculosis</td>
<td>➤ Lymph node tuberculosis</td>
</tr>
<tr>
<td>➤ Meningitis tuberculosis</td>
<td>➤ Peripheral joints tuberculosis</td>
</tr>
<tr>
<td>➤ Peritoneal tuberculosis</td>
<td>➤ Skeletal tuberculosis</td>
</tr>
<tr>
<td>➤ Intestinal tuberculosis</td>
<td></td>
</tr>
<tr>
<td>➤ Spinal tuberculosis</td>
<td></td>
</tr>
<tr>
<td>➤ Genital tract tuberculosis</td>
<td></td>
</tr>
<tr>
<td>➤ Urinary tract tuberculosis</td>
<td></td>
</tr>
<tr>
<td>➤ Bilateral pleurisy</td>
<td></td>
</tr>
</tbody>
</table>

*Revised National Tuberculosis Control Programme (RNTCP)

1.1. ABDOMINAL TUBERCULOSIS

Abdominal TB is defined as TB infection of the abdomen including gastrointestinal tract, peritoneum, omentum, mesentery and its nodes and other solid intra-abdominal organs like liver, spleen and pancreas. It is one of the most common forms of EPTB. It may be caused by *Mycobacterium tuberculosis*, *Mycobacterium bovis* and atypical mycobacteria.

Classification of the abdominal TB-

1. Gastrointestinal tuberculosis
   1.1. Ulcerative
   1.2. Hypertrophic or hyperplastic
   1.3. Sclerotic or fibrous
   1.4. Diffuse colitis

2. Peritoneal tuberculosis (Tuberculosis of the peritoneum)
   2.1. Acute tuberculosis peritonitis
   2.2. Chronic peritoneal tuberculosis
   2.2.1. Ascetic form
   2.2.2. Encysted form
   2.2.3. Fibrous form
   2.2.3. a. Adhesive type
   2.2.3. b. Plastic type

3. Tuberculosis of the mesentery and its content
3.1. Mesenteric adenitis
3.2. Mesenteric cyst(s)
3.3. Mesenteric abscess(es)
3.4. Bowel adhesions
3.5. Rolled-up omentum

4. Tuberculosis of the solid viscera
   4.1. Liver, biliary tract and gall bladder
   4.2. Pancreas
   4.3. Spleen

5. Miscellaneous- Retroperitoneal lymph node tuberculosis, etc.

1.1.1. Gastrointestinal Tuberculosis (GITB)

In India about 3-20% of GITB is due to intestinal obstruction and about 5-7% of all gastrointestinal perforations have been reported to be due to TB. GITB can occur primarily or it can be secondary to a TB focus elsewhere in the body. Infection more often reaches to the abdomen by (a) swallowing of infected sputum containing the bacilli; (b) haematogenous dissemination from a focus of active PTB, miliary TB, or silent bacteraemic phase of primary TB; and (c) spread of the disease from infected adjacent viscera.

1.1.1. A. Tuberculosis of small bowel and colon

Any region of the GIT from mouth to anus can be affected by TB. Ileocaecal area is commonly affected site due to the abundance of lymphoid tissues (Peyer’s patches). Increased physiological stasis, increased rate of fluid and electrolyte absorption and minimal digestive activity permitting greater contact time between the organism and the mucosal surface in the ileocaecal area renders this region more vulnerable to the development of intestinal TB. The vulnerability of ileocaecal region has also been contributed directly to Peyer’s patches and the associated M-cells. Often, intestinal lesion starts as crypt abscess, followed by infection of Peyer’s patches. As the disease progresses, the lymphoid follicles become infiltrated and inflammation extends throughout the submucosa. Eventually the epithelial layer above the Peyer’s patches ulcerate giving rise to the characteristic histopathologic appearance of ulcerative TB enteritis. GITB can be ulcerative, hypertrophic, ulcerohypertrophic, diffuse colitis and sclerotic forms. Abdominal pain is the most common symptom. The pain is most commonly located in right lower quadrant of the abdomen. The pain particularly with intestinal obstruction has a cramp-like or colicky character. Pain may be
diffuse or dull in character especially when the peritoneum or mesenteric lymph nodes are involved. Sometimes, pain may be severe. Diarrhoea with mucus occurs in 11-20% of the patients. Weight loss, anorexia, nausea, vomiting, constipation may also be noticed. Fever and menstrual abnormality also encountered in some patients.

1.1.1. B. Oesophageal tuberculosis

TB of oesophagus is very rare. Oesophageal involvement usually occurs due to direct extension of infection from adjacent affected structures such as mediastinal lymph nodes or the lung. Upper part of the oesophagus is more often involved than the lower part.

1.1.1. C. Gastric tuberculosis

Gastric TB is also rare, due to the presence of gastric acid and the paucity of lymphoid tissue in stomach. Ulcerative form is the commonest form of GITB.

1.1.1. D. Duodenal tuberculosis

It is also a rare form of GITB, usually with obstructive or non-specific dyspeptic symptoms. Obstruction is more often due to extrinsic compression and it is caused by lymph nodes or adhesions.

1.1.1. E. Tuberculosis of appendix

It is fairly common in patients with active ileocaecal TB, but the incidence of isolated TB of the appendix is rare even in area where TB is highly endemic.

1.1.1. F. Anal tuberculosis

Anal and perianal TB are rare but pose diagnostic problems clinically. They are mostly ulcerative.

1.1.2. Peritoneal Tuberculosis

The risk of peritoneal tuberculosis is greater in patients with HIV infection or cirrhosis and in those undergoing continuous ambulatory peritoneal dialysis. Peritoneal tuberculosis results from reactivation of latent foci in the peritoneum. Patients present with the insidious onset of ascites, abdominal pain, and fever. Peritoneal fluid is exudative. Peritoneal biopsy guided by laparoscopy or mini-laparotomy can be diagnostic in more than 95%.
1.1.3. Investigations of Abdominal Tuberculosis

Abdominal TB can be investigated by following examinations –

1. Haematology and serum biochemistry
2. Mantoux test
3. Imaging studies
   - Chest radiography
   - Plain X-ray abdomen
   - Barium studies
   - Abdominal ultrasonography
   - Abdominal computerized tomography
4. Serodiagnosis
5. Polymerase chain reactions
6. Scintigraphy
7. Endoscopy
8. Fine needle aspiration cytology
9. Peritoneal biopsy
10. Laparoscopy

1.1.4. Treatment Strategy

Presently the treatment of tuberculosis is carried out as follows:

- **Accurate care:** Isolation of all patients with active pulmonary/extrapulmonary tuberculosis and continued isolation until combined drug treatment has been administered for two weeks and three consecutive sputum smears have tested negative.
- Hospitalization of patients with severe pulmonary and extrapulmonary tuberculosis for initial treatment.
- **Medical therapy:**

  For patients with pulmonary and extrapulmonary tuberculosis (excluding children and pregnant women) 4 to 9 month combination regimen of first line antitubercular agents is used (CDC, 1994).

  - Use of second line agents if resistance to first line agents occurs, sputum culture of patients remain positive after three months or acid fast bacilli smears remain positive.
after five months of tuberculosis therapy suggesting patient's resistance to first line agents (Raviglione and O'Brian, 1998).

- However shortened antitubercular drug regimens (> 4 months) are associated with higher relapse rates (Holmes and Garner, 1999).
- Non-responders having multi-drug resistant tuberculosis need to be treated with extended regimens of 12-18 months (Lordi and Reichman, 1999).
- Invasive approaches in cases with extrapulmonary tuberculosis with severe clinical manifestations like skeletal TB, tuberculosis meningitis.

The antitubercular drugs used in the treatment of tuberculosis are generally classified as first and second line agents based on the extent of use. Table 1.2 lists the first line agents used in the treatment along with their recommended doses. The first line agents like isoniazid and pyrazinamide that combine good efficacy with acceptable level of toxic side effects, are most widely used. Second-line agents, such as ethionamide and p-aminosalicylic acid, are also used. Isoniazid is regarded as a primary drug in the treatment of tuberculosis. In many respects it is an ideal agent being bactericidal, relatively nontoxic, orally active and inexpensive. It is bacteriostatic for resting bacilli. It can kill both intracellular and extracellular organism which are rapidly dividing. Emergence of isoniazid resistant strains of *M. tuberculosis* is a frequent (10 % incidence in India) problem. One bacillus in $10^6$ bacilli is genetically resistant to isoniazid. So there is a fair chance of selection of these resistant bacteria with subsequent dissemination in the population at large.

**Table 1.2. List of essential antitubercular drugs**

<table>
<thead>
<tr>
<th>Essential anti-TB drugs</th>
<th>Mode of action</th>
<th>Recommended dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Daily 3×/WK 2×/WK*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3×/WK 2×/WK*</td>
</tr>
<tr>
<td>Isoniazid (H)</td>
<td>Bactericidal</td>
<td>5 (4-6) 10 (8-12) 15 (13-17)</td>
</tr>
<tr>
<td>Rifampicin (R)</td>
<td>Bactericidal</td>
<td>10 (8-12) 10 (8-12) 10 (8-12)</td>
</tr>
<tr>
<td>Pyrazinamide (Z)</td>
<td>Bactericidal</td>
<td>25 (20-30) 35 (30-40) 50 (40-60)</td>
</tr>
<tr>
<td>Ethambutol (E)</td>
<td>Bacteriostatic</td>
<td>15 (15-20) 30 (25-35) 45 (40-50)</td>
</tr>
</tbody>
</table>

*WHO does not generally recommend twice weekly regimen. 3×/WK and 2×/WK signify thrice weekly and twice weekly regimen.*
Isoniazid is rapidly and completely absorbed after oral administration. Peak blood concentration after oral administration occurs one to two h after administration. A usual dose of 5 mg/kg body weight produces a peak concentration of approximately 3-5 µg/mL, which is 60-100 times the MIC (0.05 µg/mL) of the drug. Food and antacids interfere with its absorption. Penetration into different tissues and body fluids, including CSF is good, producing concentration similar to those found in the serum. The commonly recommended daily dose of isoniazid is 5 mg/kg in adults as well as in children. Vitamin B₆ (pyridoxine) should be administered daily (10-15 mg) to prevent adverse reaction in adults and pregnant women.

EPTB is paucibacillary and any treatment regimen effective for pulmonary TB is also effective for the treatment of EPTB. In study on spinal TB and abdominal TB the role of surgery is also addressed. The treatment of EPTB follows standard RNTCP treatment guidelines depending on categorization and is consistent with international recommendations by WHO and the International Union against Tuberculosis and Lung Disease (IUATLD) (WHO, 2003, IUATLD, 2000). Categorization is done according to patients' history, clinical and diagnostic criteria. Patients that seriously suffering with EPTB, are treated according to RNTCP category I regimen, which include initially isoniazid (H), rifampicin(R), pyrazinamide (Z) and ethambutal (E) for two month, given thrice a week. After two-month intensive phase a four-month continuation phase is started which consists of isoniazid and rifampicin, given thrice a week (Table 1.3). Streptomycin is given instead of ethambutal in TB meningitis patients. In this case continuation phase of treatment is extended for six to seven months. Initially in hospitalized patients of meningitis and pericarditis TB steroids should be given, which reduced gradually over six to eight weeks. If patients are not seriously suffering with EPTB, they should be treated with the RNTCP Category III regimen that consists of isoniazid, rifampicin, pyrazinamide for two months given thrice a week (intensive phase), then a four months continuation phase of isoniazid and rifampicin is given thrice a week. Dosage information for the adults and children are given in Table 1.4 and 1.5.

However, concern has been expressed on poor bioavailability of rifampicin from fixed dose combination (FDC) product containing isoniazid and/or pyrazinamide. The WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) issued a joint statement in 1994 pointing out that antitubercular FDC products should only be used if the bioavailability of at least the rifampicin component has been demonstrated (WHO, 1993; WHO, 1994). Subsequently, a collaborative effort known as ‘The Fixed Dose Combination Project’ was launched.
Table 1.3. WHO recommended RNTCP treatment regimens for TB

<table>
<thead>
<tr>
<th>Tuberculosis treatment</th>
<th>Tuberculosis patients</th>
<th>Initial phase</th>
<th>Continuation phase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category I</strong></td>
<td>New smear positive PTB; new smear negative PTB with extensive parenchymal involvement; new cases of severe form of EPTB</td>
<td>2 HRZE (2 HRZS) 2H3R3Z3E3 (2H3R3Z3S3)</td>
<td>6 HE 4 HR 4 H3R3</td>
</tr>
<tr>
<td><strong>Category II</strong></td>
<td>Sputum smear positive relapse; treatment failure; treatment after interruption.</td>
<td>2 SHRZE / 1HRZE 2 S3H3R3Z3E3 / 1 H3R3Z3E3</td>
<td>5 H3R3E3 5 HRE</td>
</tr>
<tr>
<td><strong>Category III</strong></td>
<td>New smear negative PTB (other than in category I); new less severe forms of EPTB.</td>
<td>2 HRZ 2 H3R3Z3</td>
<td>6 HE 4 HR 4 H3R3</td>
</tr>
</tbody>
</table>

The number before the letters refers to the number of months of treatment. The subscript refers to the number of doses per week.

Table 1.4. Dosage information of anti-TB drugs for adult patients

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (thrice weekly)</th>
<th>Number of pills in combipack</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (H)</td>
<td>600 mg</td>
<td>2</td>
</tr>
<tr>
<td>Rifampicin (R)</td>
<td>450 mg*</td>
<td>1</td>
</tr>
<tr>
<td>Pyrazinamide (Z)</td>
<td>1500 mg</td>
<td>3</td>
</tr>
<tr>
<td>Streptomycin (S)**</td>
<td>1200 mg</td>
<td>3</td>
</tr>
<tr>
<td>Ethambutol (E)</td>
<td>750 mg</td>
<td>-</td>
</tr>
</tbody>
</table>

* Patients with 60 kg or more in weight are given an extra dose of 150 mg of Rifampicin.
** Patients over 50 years of age are given 300 mg of streptomycin.

WHO does not recommend twice weekly regimen. 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 patients were receiving thrice weekly or daily regimen. There is therefore a bigger risk of treatment failure. TB treatment programs are based on extent of the disease determined smear tests. Table 1.3 gives the treatment regimen for each category.
Table 1.5. Dosage information of anti-TB drugs for children

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Daily regimen</th>
<th>Thrice weekly regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (H)</td>
<td>5 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Rifampicin (R)</td>
<td>10 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Pyrazinamide(Z)</td>
<td>25 mg/kg</td>
<td>35 mg/kg</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>15 mg/kg</td>
<td>15 mg/kg</td>
</tr>
<tr>
<td>Ethambutol (E)</td>
<td>15 mg/kg</td>
<td>30 mg/kg</td>
</tr>
</tbody>
</table>

* Should be avoided to children below 6 years of age

As a part of this exercise, a protocol was established for bioequivalence testing of rifampicin from FDC products (Fourie et al., 1999). Recently the DOTS (Directly Observed Therapy Strategy) have been introduced by WHO to ensure regular and uninterrupted supply of FDC product.

The deficiency in the delivery of proper dose of rifampicin has serious implications, as it is known that doses of the drug less than 9 mg/kg body weight can result in therapeutic failure and hence can be the cause of development of drug resistance (Long et al., 1979). A set of studies indicates that rifampicin bioavailability is found to decrease in the presence of isoniazid when the drugs are released in the gastric medium.

Although, a number of reasons have been cited for this interaction, the most critical one is a chemical reaction between the metabolite of rifampicin with isoniazid under the acidic conditions leading to reduced drug absorption and decreased bioavailability (Singh et al., 2001). The interaction has been studied in simulated in vitro conditions. Steps need to be undertaken to find a solution to this problem. An extensive literature survey has suggested the following ways to reduce this interaction in the gastric medium.

- Segregation of the site of release of the two drugs; rifampicin in the gastric medium and isoniazid in the small intestine.
- Use of alkalinizers at the time of administration of FDC products.
- Enteric coating of the solid formulations.

The present study is based on the segregation of drug delivery of isoniazid and rifampicin to avoid the interaction in the gastric medium using micro-particulate technology. Formulation of microspheres will also results in sustaining the drug level in plasma resulting in reduced dose of the drug and decreased side effects.
1.2. PARTICULATE DRUG CARRIER

Particles currently used for drug delivery are of two types

1. Nanoparticles ranging in size from 10-1000 nm and
2. Microparticles ranging in size from 1-1000 μm.

Nanoparticles

They are usually prepared from synthetic materials but they can also be prepared from other macro molecular materials such as proteins. Polymeric nanoparticles can be prepared either by polymerization of monomers or preformed polymers. For drug delivery purposes biodegradable polymers are preferred: poly (alkyl-cyanoacrylate), poly (lactide), poly (DL-lactide-co-glycolide) are example of such materials. Albumin, gelatin and chitosan can be modified chemically or physically in order to obtain stable, water insoluble colloid; heat denaturation or salt desolvation followed by covalent cross-linking. Drug incorporation can be done by supercritical adsorption or by embedding in the matrix, leading to different release time profile and mechanism (Devisaguet et al., 1992). There are some examples relating to use of nanoparticles for gastrointestinal application- vincamine (Maincent et al., 1986), antigens (Eldridge et al., 1990), indomethacin (Ammoury et al., 1991), lipoidol (Damge et al., 1991) and insulin (Couvreur et al., 1980).

Microspheres

Microspheres refer to a particle with a size range of 1-1000 μm, within the broad category of microparticles, ‘microspheres’ specifically refers to spherical microparticles and the sub category of ‘microcapsules’ applies to microparticles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid or even gas.

1.2.1. Biodegradable Microspheres as Oral Sustain Released Drug Delivery System

Biodegradable microparticulate carriers are of interest for oral delivery of drug to

- Improve the bioavailability
- Enhanced drug absorption
- Target particular organ and reduce toxicity
- Improve gastric tolerance of gastric irritant substances to stomach and
- As carrier for antigens
Conceptually, the phospholipid bilayer of plasma membrane of the epithelial cells that normally line the intestine (the enterocytes) is considered to be the major factor restricting the free movement of substances from the lumen to blood stream. The term ‘persorption’ was suggested by Volkheimer to allow the passage of particles upto 100 µm in diameter (Volkheimer and Schultz, 1968), which subsequently reaches the portal venous blood and thoracic lymph. Although this subject is of considerable controversy, new strategy has been developed similar to the ‘Trojan horse’. The active molecules to be delivered are hidden inside hydrophobic, biodegradable microspheres that can be taken up by endocytosis by intestinal cells. The extent and pathway of particles uptake is different in different part of the intestine. The M cell of the payer’s patches represent a lymphatic island within the intestinal mucosa and possibly the major gateway through which particles can be absorbed. Table 1.6 gives the various sites of particle uptake based on the particle size.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Size range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterocyte or endocyte (RES system)</td>
<td>&lt; 220 nm</td>
</tr>
<tr>
<td>Paracellular uptake</td>
<td>100 nm-200 nm</td>
</tr>
<tr>
<td>Intestinal macrophage</td>
<td>1µm</td>
</tr>
<tr>
<td>Persorption</td>
<td>5 µm-150 µm</td>
</tr>
<tr>
<td>Payer’s patches</td>
<td>20 nm-10 µm</td>
</tr>
<tr>
<td>Follicle associated epithelium</td>
<td>&lt; 750 nm</td>
</tr>
</tbody>
</table>

Peroral drug delivery may be further enhanced by addition of mucoadhesive substances to microparticles with subsequent longer interaction of the particles with the cell membrane; an alternative strategy to increase the interaction of the particles with target cell is to mix them with lipid delivery vehicles, such as lecithin. It should be pointed out that the term ‘uptake’ of particles for gut tissue may include both adsorbed particles (particle that remain on the surface of the intestinal cells) and absorbed particle (particles that are actually translocated to the blood stream) and are therapeutically relevant (Fasano, 1998).

1.2.2. Prerequisites for Ideal Microparticulate Carriers

The material utilized for the preparation of microparticulate system should fulfill the following prerequisites:
1.2.3. Material(s) Used for The Preparation of Microparticulate System

A number of different substances both biodegradable as well as non-biodegradable had been investigated for the preparation of microspheres. These materials include polymers of natural and synthetic origin and modified natural substances.

Natural polymers
- Carbohydrates (Starch, Agarose, Chitosan, Gellan gum, Alginate).
- Chemically modified carbohydrates (HPMC, HPEC, Ethyl cellulose, Poly (acryl) starch, Poly (alkyl) dextran).
- Proteins (Albumin, Gelatin, Collagen).

Synthetic polymers
- Biodegradable (Poly (lactic acid), Poly (lactide G), Poly (DL-lactide-co-glycolide), Poly (e-caprolactone), Polyanhydrides).
- Non-biodegradable (Eudragit L, Eudragit RS, Eudragit RL, Polymethyl methacrylate, Epoxy polymers).

1.2.4. General Methods of Preparation

The microspheres can be prepared by using any of the several techniques discussed in the following section, but the choice of the technique mainly depends on the nature of the polymer used, the drug, the intended use and the duration of therapy. Moreover, the method of preparation and its choice are equivocally determined by some formulation and technology related factors.

- Single emulsion technique
Chapter 1

Introduction

- Double emulsion technique
- Phase separation coacervation method
- Spray drying and spray congealing
- Solvent extraction or solvent evaporation technique
- Ion gelation method

Certain formulation and technology related factors govern the choice of the technique being utilized:
- The particle size requirement.
- The drug or the protein should not be adversely affected by the process.
- Reproducibility of the method and release profiles.
- No stability problem.
- No toxic product to be associated with final product.

1.2.4.A. Single emulsification method

The microparticulate carriers of natural polymers, i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium.

This method utilizes the reactive functional group of polymer to cross-link with aldehyde group of cross linking agent. In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the aqueous polymer solution in the oil phase. Aqueous droplets are stabilized by using suitable surfactant. The stable emulsion is cross linked by using appropriate cross linking agent such as gluteraldehyde to harden the droplets. Microspheres are filtered and washed repeatedly with n-hexane followed by alcohol and then dried.

The cross linking may also be achieved by means of heat. Cross linking by heat is affected by adding the dispersion to previously heated oil. Heat denaturation is however, not suitable for the thermolabile drugs while the chemical cross linking suffers disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation.

Drawback of single emulsion method
- Tedious procedure
- Use of harsh cross linking agents, which might possibly induce chemical reaction with the active agent.
Complete removal of un-reacted cross linking agent may be difficult in this process.

1.2.4.B. Double emulsification method

The technique involves the formation of multiple emulsion or double emulsion of type w/o/w and is best suited to the water soluble drugs, proteins, peptides and the vaccines.

1.2.4.C. Phase separation method

Phase separation method is specially designed for preparing the reservoir system, i.e. to encapsulate water soluble drugs e.g. peptides, proteins, however, some of the preparation are of matrix type particularly, when the drug is hydrophobic in nature e.g. steroids.

1.2.4.D. Spray drying and spray congealing

This method is based on drying of atomized droplets in a stream of air. In this method, polymer is first dissolved in volatile organic solvent, drug is then dissolved or dispersed in the solution and then, a suitable cross linker is added. This solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of free flowing particles.

1.2.4.E. Ionic gelation method

This is a simple and mild process in which reversible physical cross-linking of polymers by electrostatic interaction occurred between cations (usually divalent) and functional groups of polymers. Microspheres made of gel-type polymers, such as alginate, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing microdroplets which fall into hardening bath containing polyvalent cations (usually calcium chloride solution), whereby the divalent calcium ions cross-link the polymer forming the gelled microspheres. The surface of the microspheres further can be modified by coating them with polycationic polymers, like chitosan or polylysine after fabrication.

1.2.4.F. Solvent extraction or solvent evaporation method

Solvent extraction method involves removal of organic solvent by extraction of water miscible organic solvent such as isopropanol resulting in hardening of microspheres.
1.2.5. Drug Loading in Microparticulate System

The drug loading in the microspheres takes place by two methods.

- Loading in the preformed microspheres
- Loading during the preparation (i.e. in situ loading)

The active ingredient can be loaded by means of the physical entrapment, chemical linkage and surface adsorption. While maximum loading is observed during preparation of microspheres, it may be affected by process variables such as method of preparation, presence of additives (i.e. cross-linker, surfactant etc.), and agitation intensity etc. Percent incorporation is relatively less in preformed microspheres but the major advantage is the absence of effect of process variables.

1.2.6. Characterization of Microparticulate Systems

Microparticulate systems possess different microstructures depending on their method of preparation and condition during preparation. These microstructures determined the release property and stability of the carrier (Malmstern, 2002).

1.2.6.A. Particle size and shape

Shape of the particulate systems can be determined by light microscopy, scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM). Particle size distribution can be measured by laser light scattering and multisize coulter counter.

1.2.6.B. Entrapment efficiency

Entrapment efficiency can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents according to monographic requirement.

1.2.6.C. Release study

Release study of microspheres is carried out in different buffer solutions by using rotating paddle or basket apparatus or by using dialysis method. In case of paddle/basket type sample is agitated at specific rpm. Samples are taken at specific time interval and replaced by same amount of dissolution media. In dialysis method the microspheres are kept in dialysis...
bag. Sample of dialysate are taken at regular time interval and replaced by same amount of dissolution media.

1.2.6.D. Drug release kinetics

The release profile from the microspheres is cumulative effect of the nature of the polymer used in the preparation as well as nature of the drug. The kind of drug, its polymorphic form, crystallinity, particle size, solubility and amount in the pharmaceutical dosage form can influence the release kinetics. Thus a water soluble drug incorporated in the matrix is mainly released by diffusion, while for a low water soluble drug the self erosion of the matrix is the principle release mechanism. The release of drug from both biodegradable as well as non-biodegradable microspheres is influenced by the structure or micromorphology of the carrier. Some theoretically possible mechanism may be considered for the release of drug from microparticles

- Liberation of drug due to polymer erosion or degradation
- Self-diffusion through the pores
- Release from the surface of the polymer
- Pulse delivery initiated by the application of an oscillating or sonic field

Several kinetic models have been proposed to describe the drug release kinetics from modified release dosage form (Costa and Lobo, 2001). These kinetic models may include, Zero order, First order, Higuchi model, Hixon-Crowell model and Baker and Lonsdale model.

- Zero order kinetics:

The pharmaceutical dosage forms following zero order kinetics, release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a prolonged action (Varelas et al., 1995). For this model, a graph of drug-dissolved fraction versus time will be linear. The following relation, in a simple way can express this model.

\[ Q_t = Q_0 + K_0t \]

Where, \( Q_t \) is the amount of drug dissolved in time \( t \), \( Q_0 \) is the initial amount of drug in the solution (most times \( Q_0 = 0 \)) and \( K_0 \) is the zero order release rate constant.
❖ **First order kinetics:**

The pharmaceutical dosage forms following this kinetics profile, such as those containing water-soluble drugs in porous matrices, release the drug in a way that is proportional to the amount of drug remaining in the interior, in such way that amount of drug released by unit of time diminishes (Mulye and Turco, 1995). Mathematically this model can be expressed as,

\[ \log Q_t = \log Q_o + \frac{K_t}{2.303} t \]

Where, \( Q_t \) is the amount of drug released in time \( t \), \( Q_o \) is the initial amount of drug in the solution and \( K_t \) is the first order release rate constant. In this way, a graphic of the decimal logarithm of the released amount of drug versus time will be linear.

❖ **Higuchi model:**

Higuchi developed several theoretical models to study the release of water-soluble and low water-soluble drugs incorporated in semi solid and/or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. To study the dissolution from a planar system having a homogeneous matrix, the relation obtained, was as follows,

\[ Q = \sqrt[3]{D} (2C - C_o)C_s t \]

Where, \( Q \) is the amount of drug released in time \( t \) per unit area, \( C \) is the total drug concentration, \( C_o \) is the drug solubility in matrix, and \( D \) is the diffusivity of the drug molecules (diffusion constant) in the matrix. In a general way, it is possible to resume the Higuchi model to the following expression (generally known as the simplified Higuchi model)

\[ Q = K_h t^{1/2} \]

Where, \( K_h \) is the Higuchi dissolution rate constant. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms (Higuchi, 1963).

❖ **Hixon-Crowell cube root model:**

The Hixon-Crowell cube root law describes the release from system where there is a change in surface area and diameter of the particles or microspheres during drug release process (Hixon and Crowell, 1993; Abdou, 1989).
Mathematically this model can be expressed as,

$$W_0^{16} - W_t^{16} = k_{HC} t$$

Where $W_0$ is initial drug load at time zero taken as 100%, and $W_t$ is percentage drug undissolved at time $t$.

❖ **Baker and Lonsdale model:**

Baker and Lonsdale described the release rate of a drug from a spherical matrix as follows (Baker and Lonsdale, 1987; Karajgi et al., 1993):

$$1.5[1-(1-F)^{0.66}] - F = k_{HC} t$$

Where, $F$ is the fraction of drug released at time $t$' and $k_{HC}$ is the release rate constant.

Fitness of the data into various kinetic models can be assessed by determining the correlation coefficient and the rate constants, for respective models.

1.3. GASTRORETENTIVE DRUG DELIVERY SYSTEMS

Gastroretentive drug delivery systems, also termed as hydrodynamically balanced or floating drug delivery systems, are suitable for those drugs that act locally in the stomach, primarily absorbed in the stomach, poorly soluble at an alkaline pH, have a narrow window of absorption, and are unstable in the intestinal or colonic environment (Singh and Kim, 2000). To provide good floating behavior in the stomach, the density of the device should be less than that of the gastric contents. Both single and multiple unit floating systems have been developed. The single-unit floating systems are more popular but they have 'all-or-none' emptying property causing high variability in gastrointestinal transit time (Whitehead et al., 1998, Talukder and Fassihi, 2004). The multiple-unit floating dosage forms are better accepted due their reduced intersubject variability in absorption and minimized chances of dose dumping (Rouge et al., 1997). These dosage forms distributed widely throughout the gastrointestinal tract, holding the possibility of an extended and more reliable release of the drug from the systems (Sato et al., 2003). Intragastric residence positions of nonfloating and floating units are depicted well in Figure 1.1. Both natural and synthetic polymers have been used to prepare floating microspheres.
1.3.1. Factors Affecting Gastric Retention of Dosage Forms

Factors that affect gastric retention time of dosage form may include density and size of the dosage form, nature of the food, food intake, age, sex, sleep, posture, disease state of the individual and administration of drugs.

1.3.1.A. Density of dosage form

For the purpose of flotation, the density of the dosage forms should be lower than that of gastric fluid. A density of less than 1.0 gm/cm$^3$ is needed to exhibit floating property of formulation. Usually floating tendency of the dosage form decreases with time, because as a result of the development of hydrodynamic equilibrium the dosage form may immersed into the gastric fluid (Timmermans and Moes, 1990).

1.3.1.B. Size of dosage form

The size of the dosage form may also affect the gastric retention time. Mostly larger sized dosage forms will retain more in stomach because the larger size would not allow the dosage form to quickly pass through the pyloric antrum into the intestine (El-Kamel et al., 2001).

1.3.1.C. Intake of food

The presence or absence of food in the stomach affect the gastroretention time of the dosage form. Generally, the presence of food increases the gastroretention time of the...
formulation. In a reported study, misoprostol bilayer floating capsule showed the mean retention time about 199 ± 69 minutes; but after a light food intake it was increased up to 618 ± 208 minutes (Oth et al., 1992). Whitehead et al. also supported above results by their experiments in which he showed an increase in the relative heights of the floating dosage forms after food administration (Whitehead et al., 1998).

1.3.1. D. Effect of gender, posture and age

A study reported that females have comparatively shorter mean ambulatory gastroretention time as compare to males. The authors also studied the effect of posture on gastric retention time, and found no significant difference in the mean gastric retention time for individuals in upright, ambulatory and supine state (Mojaverian et al., 1988). The effect of posture on the retention time was studied by Gansbeke et al., 1991. He reported that in the upright position, the floating systems floated to the top of the gastric contents and remained for a longer time, as compared to non-floating systems. But in supine position, these systems (floating systems) showed faster emptying than non-floating systems of same size (Timmermans and Andre, 1994).

1.3.2. Classification of Gastroretentive Drug Delivery Systems

1.3.2. A. Bioadhesive systems

These types of drug delivery systems are used for local delivery of drugs within the lumen to enhance their absorption at the specific site. This approach involves the use of bioadhesive polymers, which can adhere to the epithelial surface of the stomach. Materials that bind to the biological substrates, such as mucosal membrane are known as bioadhesive/mucoadhesive substances. Bioadhesiveness offers the possibility of creating an intimate and prolonged contact of material at the site of administration. That causes enhanced absorption with a controlled release of drug and it also improve patient compliance by reducing the frequency of administration. The bioadhesive properties of mucin have been utilized for the development of hydrodynamically balanced drug delivery systems (Chickering et al., 1995). The forces of interaction between the gastrointestinal mucosa and the individual polymers can be measured by a microbalance based system. The adherence of the delivery systems can be studied by using Cahn Dynamic Contact Angle Analyzer (Ikeda et al., 1992). Some of the most commonly used excipients in these systems include sodium alginate, ispaghula husk, jack-fruit latex, carbopol, chitosan, carboxymethyl cellulose, lectins, gliadin,
polycarbophil, etc. Various researchers have worked on delivery systems having both floating and bioadhesiveness characteristics.

1.3.2. B. High-density systems

Sedimentation phenomena may be utilized to enhance the residence time of the delivery systems in the stomach. Depending on the density of the particles, the GI transit time of the system can be extended up to 6–24 h. Dense particles may trap in rugae also tend to withstand the peristaltic movements of the stomach wall. Commonly used excipients that are used to increase the density of the system are titanium dioxide, iron powder, barium sulphate, zinc oxide, etc.

1.3.2. C. Large single unit dosage forms

Large single unit dosage forms may also be used to increase the residence time of the delivery systems. They are larger in size than pyloric opening and could not cross through it, so, retained in the stomach. But these types of systems may cause some problems like gastroplasty, intestinal adhesion and bowel obstruction.

1.3.2. D. Swelling systems

These systems when come in contact with gastric fluid get swelled to a size that they cannot pass through the pylorus; as a result, the dosage form is retained in the stomach for a longer period of time (Singh, 2000).

1.3.2. E. Floating systems

Floating systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period. While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased gastro-retention time and reduces fluctuation in plasma drug concentration (Singh, 2000).

1.3.3. Approaches Used to Design Floating Systems

Various approaches have been used for designing single and multiple units floating systems (Yang and Fassihi, 1996).


Chapter 1: Introduction

Single-Unit dosage forms

Low density approaches, in this system globular shells that have lower density than gastric fluid are used as a drug carrier. A fluid-filled system that floats in the stomach may also be used to obtain a buoyant dosage form. Coated shells, in these types of systems popcorn, poprice, and polystyril have been used as drug carriers. These shells are undercoated with polymeric materials such as methacrylic polymer and cellulose acetate phthalate. Then they are further coated with a drug-polymer mixture (Burns et al., 1998). Fluid-filled floating chamber, in this approach, a gas-filled floating chamber is incorporated into a microporous component encasing a drug reservoir (Joseph et al., 2002). Self-correcting floatable asymmetric configuration drug delivery system, in this type of systems a disproportionate 3-layer matrix technology is used to control the drug release from the formulation with zero-order release kinetics. The system was designed in such a manner that it floated in the gastrointestinal tract for a prolong period of time causing maximum absorptive capacity and greater bioavailability. This characteristic is suitable for the drugs having pH-dependent solubility, a narrow window of absorption, and that are absorbed actively from the proximal or distal portion of the small intestine.

Multiple-Unit dosage forms

Multiple-unit dosage forms are developed in such a way that they have all the advantages devoid of disadvantages of a single-unit dosage forms. In this type of systems microspheres, spherical polymeric microsponges have been prepared by using various polymers such as albumin, gelatin, starch, polymethyl methacrylate, polyacrylamine, and poly (alkyl cyanoacrylate).

Floating drug delivery systems remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time due to having a bulk density less than gastric fluids. The drug is released slowly at the desired rate from the system while they are floating on the gastric content. After release of drug, the residual system is emptied from the stomach. This causes an increased gastrointestinal retention time and a better plasma drug concentration. For the proper buoyancy of the delivery system, a minimal level of floating force (F) is also required to keep the dosage form continuous buoyant on the surface of the meal. The system will better float if the floating force is more positive. Floating force (F) was given by the vectorial sum of buoyancy \( F_{(b)} \) and gravitational forces \( F_{(g)} \) acting on the test object.

\[
F = F_{(b)} - F_{(g)}
\]
Above equation can be rewritten as,

\[ F = (d_f - d_s)gV = (d_f - W/V)gV \]

Where F is the resultant weight of the object, \( d_f \) and \( d_s \) represent the fluid density and solid object density, g is the acceleration due to gravity and \( W \) and \( V \) are the weight and volume of the test objects. It can be seen from above equation that if the resultant weight is more positive, better floating is exhibited by the object (Timmermans, 1994). The overall force that the delivery system is subjected can also be given by

\[ F = (\rho_m - \rho_c) gV_c \]

Where \( \rho_m \) and \( \rho_c \) are the density of floating media and test object and \( V_c \) is the volume of the test object. In this equation, two parameters, \( \rho_c \) and \( V_c \), are important for overall floating force. During the measurement of buoyancy, \( V_c \) increased due to swelling of polymer and \( \rho_c \) increased due to water uptake. This increase led to an upward rise in floating force curve, which reached a maximum (\( F_{\text{max}} \)) and declined until an equilibrium was reached (Li et al., 2002).

1.3.4. Evaluation and Characterization of Gastroretentive Drug Delivery Systems

Hydrodynamically balanced drug delivery systems are characterized by evaluating their micromeritic properties such as floating duration, dissolution profiles, specific gravity, content uniformity, hardness, friability, tapped density, particle size, true density, compressibility index and flow properties (Martin et al., 1991). They are also characterized by differential scanning calorimetry (DSC) and surface morphology. True density is determined by liquid displacement method; tapped density and compressibility index are calculated by measuring the change in volume using a bulk density apparatus. Particle size is measured by using an optical microscopy and mean particle size was calculated with the help of calibrated ocular micrometer. Angle of repose is determined by fixed funnel method (Martin et al., 1991, Umamaheshwari et al., 2003). Tapped density and compressibility index can be calculated by using following formulae-

Tapped density = Mass of microspheres / Volume of microspheres after tapping

\[ \text{Compressibility index (\%)} = \frac{V_b - V_t}{V_b} \times 100 \]

Where, \( V_b \) is the bulk volume and \( V_t \) is the tapped volume. The values, less than 15% indicates a powder good flow characteristics, whereas more than 25% indicate poor flow ability.
1.3.4.A. Floating behavior

Floating behaviour of the particulate drug delivery systems can be assessed by placing a weighed amount of particles in the simulated gastric fluid. Then the system was stirred for a definite time period. After that the layer of buoyant and sinked particles was separated by filtration. Particles of both types were dried in a desiccator until constant weight was achieved. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles (Jain et al., 2005). The percentage Buoyancy can be presented by the following formula-

\[ \text{Buoyancy} (%) = \frac{W_f}{W_f + W_s} \times 100 \]

Where, \( W_f \) and \( W_s \) are the weights of the floating and settled particles.

1.3.4.B. Dissolution test study

Dissolution tests are generally performed using USP dissolution apparatus. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of non-reactive material with not more than a few turns of a wire helix may be attached to the dosage units that would otherwise float. Wire helix may inhibit the swelling behavior of the swellable dosage form. To overcome this limitation a more reproducible and consistent method was developed in which the floating drug delivery system was fully submerged under a ring or mesh assembly and an increase in drug release was observed.

1.3.4.C. In vivo study

In vivo study for gastric residence time of a floating dosage form is determined by X-ray studies, roentgenography (Babu and Khar, 1990) or gamma scintigraphy (Timmermans et al., 1989).

1.3.5. Application of Gastroretentive Drug Delivery Systems

Gastroretentive drug delivery system offers several applications to improve the bioavailability of drugs having narrow absorption window in the upper part of the gastrointestinal tract. These systems work by retaining the dosage form at the site of absorption. Various applications of gastroretentive drug delivery systems are summarized below.

Jamia Hamdard
Floating drug delivery is associated with certain limitations. Drugs that irritate the mucosa, those that have multiple absorption sites in the gastrointestinal tract, and those that are not stable at gastric pH are not suitable candidates to be formulated as floating dosage forms. Floatation as a retention mechanism requires the presence of liquid on which the dosage form can float on the gastric contents. To overcome this limitation, a bioadhesive polymer can be used to coat the dosage so that it adheres to gastric mucosa (Chitnis et al., 1991) or the dosage form can be administered with a full glass of water to provide the initial fluid for buoyancy. Single unit floating capsules or tablets are associated with an 'all or none concept' of gastric retention; this problem can be overcome by formulating multiple unit systems like floating microspheres or microballoons (Rouge et al., 1997).

1.4. MUCOADHESIVE DRUG DELIVERY SYSTEM

Over the past 20 years, interaction between the fields of engineering, biology and polymer science has resulted in the development of a new class of biomaterials known as bioadhesive polymers. One major application for these materials has been found in the area of drug delivery systems. For drug delivery devices specifically designed for use in the gastrointestinal tract, it is paramount that the vehicles remain in the lumen of the absorbing organ for a period of time sufficient for the transfer of its bioactive ingredients. Polymers designed to produce strong adhesive interactions with biological tissues or secretions could be employed to keep a microsphere based drug delivery system in contact with the intestinal epithelium for an extended period of time. Phenomena pertinent to the adhesion of polymeric materials on biological surfaces have been reviewed and analyzed by various investigators (Ponchel and Duchene, 1991; Ranga Rao and Buri, 1988; Passl, 1989; Lenearts and Gurny, 1990; Gu et al., 1988; Ch'ng et al., 1985).

There are two major aspects important to the development of adhesive bonds between a polymer and gastrointestinal (GI) tissue: (1) the surface characteristics of the bioadhesive polymer; and (2) the nature of the biological material with which the polymer comes in contact.
The intestinal mucosa is comprised of 3 layers: a thin smooth muscle layer, connective tissue layer and a continuous sheet of columnar epithelial cells including enterocytes and goblet cells. Overlying the mucosa is a continuous protective coating, the mucus, which is comprised of over 95% water, plus electrolytes, proteins, lipids and glycoproteins- the latter, being responsible for the gel-like characteristics of the mucus. These glycoproteins consist of a protein core with covalently attached carbohydrate side-chains terminating in either sialic acid or L-fucose groups. The carbohydrate structure of intestinal mucous glycoproteins is similar to that of enterocyte cell membrane glycoproteins, Along with naturally produced mucus, the lumen of the GI tract harbors a variety of bacteria, microorganisms and ingested food products in various states of digestion (Spiro, 1970; Labat-Robert and Decaeus, 1979; Scawen and Allen, 1977; Pigman and Gottschalk, 1966; Horowitz and Pigman, 1977).

Adhesion of polymers to biological substrates may be achieved by: (1) physical or mechanical bonds (Ranga Rao and Buri, 1988; Kinloch, 1980); (2) primary or covalent chemical bonds (Ranga Rao and Buri, 1988); or (3) secondary chemical bonds (i.e. ionic) (Park and Robinson, 1985; Chen and Cyr, 1970). Physical or mechanical bonds result from polymer and mucin chain entanglement, polymer deposition in the folds of the mucosa, or inclusion of the mucus in the crevices of the polymer. Secondary chemical bonds consist of dispersive interactions (i.e. Van der Waals’ interactions) and stronger specific interactions, which include hydrogen bonds. Hydrophilic functional groups responsible for forming hydrogen bonds include hydroxyl (–OH) and carboxyl groups (–COOH) (Park and Robinson, 1985; Chen and Cyr, 1970).

Traditionally, three classes of polymers have been of interest for use as bioadhesives:

**Hydrophilic polymers:** These are the water-soluble polymers that swell indefinitely in contact with water and eventually undergo complete dissolution.

**Hydrogels:** These are water swellable materials, usually a cross-linked polymer with limited swelling capacity.

**Thermoplastic polymers:** These polymers include the non-erodible neutral polystyrene and the semi crystalline bioerodible polymers, which generate the carboxylic acid groups as they degrade, e.g. polyanhydrides and polylactic acid. Various synthetic polymers used in bioadhesive formulations include polyvinyl alcohol, polyanhydrides, polycarbonates, polyalkylene glycols, polyvinyl ethers, esters and halides, polymethacrylic acid, polymethyl
methacrylic acid, methylcellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and sodium carboxymethylcellulose.

Various biocompatible polymers used in bioadhesive formulations include cellulose based polymers, ethylene glycol polymers and its copolymers, oxyethylene polymers, polyvinyl alcohol, polyvinyl acetate and HYAFF (esters of haluronic acid).

Various biodegradable polymers used in bioadhesive formulations are poly(lactides), poly (glycolides), poly (lactide-co-glycolides), polycaprolactones, and polyalkyl cyanoacrylates. Polyorthoesters, polyphosphoesters, polyamides, polyphosphazenes are the recent additions to the polymers.

Many ligand molecules are often attached covalently to the surface of polymeric microspheres either to increase the strength of bioadhesion or to impart specificity to adhere to specific mucosal surfaces. Attachment of different anhydride oligomers (sebacic acid, bis (p-carboxy-phenoxy) propane, isophthalic acid, fumaric acid, maleic acid, adipic acid or dodecanedioic acid), positively charged ligands (polyethylenimine, polylysine), polyamino acids (polyaspartic acid, polyglutamic acid), partially purified fractions of mucin (Santos et al., 2000), and metal ions (calcium, iron, copper, zinc) have been explored to modify the bioadhesive properties of the polymers (Jacob and Mathiowitz, 2000). Multivalent ions, such as divalent or trivalent cations in the metal compounds generally, have the strongest affinity for the negatively charged mucin chains. The ligand affinity need not be based solely on electrostatic charge, but other useful physical parameters such as solubility in mucin or specific affinity to carbohydrate groups. The in vivo chelation of calcium and other metal ions by the polyacrylic acid-based microparticles leads to higher rates of absorption and inhibition of enzymes. Depletion of extracellular calcium may affect the integrity of the epithelial cells, causing enhanced permeability and higher rate of absorption. Many enzymes require metal ions for their action and chelation of these ions by the polymer causes inhibition of the enzymes (Kriwet and Kissel, 1996). Polyethylene glycol (PEG) has been reported to act as the adhesion promoter between polyacrylic acid and mucin by linear diffusion of the PEG chains into the polymeric networks of both mucin and the polymer (Lele and Hoffman, 2000). The release rate of indomethacin was studied from the bioadhesive drug delivery system (BDDS) based on the above said complexes. The PEGylated drug was designed to be a prodrug, which was linked by an easily hydrolysable anhydride bond. The complex was found to dissociate and dissolve at pH 7.4 forming polyacrylate sodium and releasing free drug and PEG.
Specific site directed new generation bioadhesives

The specific mucosal surfaces can be targeted using site-specific chemical agents that are anchored onto the polymeric DDS. The first generation mucoadhesive polymers lack specificity and can bind to any mucosal surface. This limits their use for fabrication of BDDS for a particular tissue. However, the development of polymers and microspheres grafted with mucus or cell-specific ligands have increased therapeutic benefit and made site-specific drug delivery possible (Table 1.7). Any ligand with a high binding affinity for mucin can be covalently linked to the microspheres with the appropriate chemistry, such as CDI (carbonyl di-imidazole) and be expected to influence the binding of microspheres. Targeting of the drugs can be achieved by using the following ligands:

- Lectins (Haas and Lehr, 2002; Gabor et al., 1997; Lehr et al., 1992)
- Bacterial adhesions (Lee et al., 2000; Haltner et al., 1997)
- Amino acid sequences
- Antibodies

The formation of bioadhesive bonds between soft tissue and these materials has been described in 3 steps: (1) wetting and swelling of the polymer to permit intimate contact with the biological tissue; (2) interpenetration of bioadhesive polymer chains and entanglement of polymer and mucin chains; and (3) formation of weak chemical bonds (Ponchel and Duchene, 1991; Duchene et al., 1988). Since water-insoluble, bioerodible materials are often hydrophobic and typically do not exhibit highly flexible chains, swelling and chain entanglement appear unlikely to occur. Hence, investigation of these materials for bioadhesive applications has been overlooked.

1.4.1 Theoretical Background
1.4.1.1. Mechanisms of bioadhesion

The mechanisms involved in the formation of a bioadhesive bond are not completely clear. In order to develop ideal bioadhesive systems it is important to gain a thorough understanding of the forces responsible for adhesive interactions. Most research in this area has focused on analyzing bioadhesive bonds between hydrogel polymers and soft tissue. In the case of hydrogels, it has been determined that several polymer characteristics are required to obtain adhesion: (1) sufficient quantities of hydrogen-bonding chemical groups (–OH and –COOH); (2) anionic groups (–COOH) (Park and Robinson, 1985; Chen and Cyr,
1970); (3) surface charges; (4) high molecular weight; (5) high chain flexibility; and (6) surface tensions which will induce spreading into the mucous layer (Peppas and Buri, 1985). These characteristics favor the formation of bonds which are either mechanical (Ranga Rao and Buri, 1988; Kinloch, 1980) or chemical in nature (Park and Robinson, 1985; Chen and Cyr, 1970). Thus far, very little research has been geared toward investigating the processes and mechanisms involved in the adhesion of hard, bioerodible materials to soft tissue.

Table 1.7. Specific ligands corresponding to the glycosyl groups on cell membranes, which can be used for targeting the bioadhesive microspheres to a specific site

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Glycosyl groups on cell membranes</th>
<th>Specific ligands</th>
<th>Specific site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mannose</td>
<td>Galanthus nivalis agglutinin (GNA)</td>
<td>Epithelial cells in stomach, caecum, and colon</td>
</tr>
<tr>
<td>2</td>
<td>N-Acetyl glucosamine</td>
<td>Wheat germ agglutinin (WGA)</td>
<td>Epithelial cells in stomach, caecum, colon and absorptive enterocytes in small intestine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycopersicon esculentum or tomato lectin (LEA)</td>
<td>Strong binding to M cells</td>
</tr>
<tr>
<td>3</td>
<td>N-Acetyl galactosamine</td>
<td>Lectin ML-1 from Viscum album</td>
<td>Endocytosed by villus enterocytes and goblet cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strong binding to epithelial cells in small intestine</td>
</tr>
<tr>
<td>4</td>
<td>Phytohaemagglutinin</td>
<td>Phaseolus vulgaris isoagglutinin</td>
<td>Surface cells of the stomach</td>
</tr>
<tr>
<td>5</td>
<td>Fucose</td>
<td>Aleuria aurentia agglutinin (AAA)</td>
<td>Specific binding and transcytosis by M cells</td>
</tr>
</tbody>
</table>

Jamia Hamdard 29
1.4.1.2. Theories on bioadhesion

Five general theories have been developed to describe the processes involved in the formation and disruption of bioadhesive bonds: the electronic (Derjaguin and Smiiga, 1966; Derjaguin, 1977), adsorption (Kinloch, 1980; Huntsberger, 1966; Huntsberger, 1967; Good, 1977; Tabor, 1977), wetting (Peppas and Buri, 1985; Helfand and Tagami, 1972a; Helfand and Tagami, 1972b), diffusion (Kinloch, 1980; Voyutskii, 1963; Mikos and Peppas, 1986), and fracture theories (Ponchel, 1987; Kaelble, 1974; Kammer, 1983). These theories have been used as guidelines in engineering possible bioadhesive systems. Some involve the formation of mechanical bonds while others focus on chemical interactions.

1.4.1.2.A. The electronic theory

The electronic theory is based on an assumption that the bioadhesive material and the glycoprotein mucin network have different electronic structures. On this assumption, it is believed that upon contact, electron transfer occurs in an attempt to balance Fermi levels, causing the formation of a double layer of electrical charge at the interface. The bioadhesive force is attributed to attractive forces across this electrical double layer (Derjaguin and Smiiga, 1966). The electronic theory has produced some controversy regarding whether the electrostatic forces are an important cause or the result of contact between the bioadhesive and the biological tissue (Derjaguin, 1977).

1.4.1.2.B. The adsorption theory

This theory states that the bioadhesive bond formed between the adhesive substrate and the intestinal mucosa is due to van der Waals' interactions, hydrogen bonds and related forces (Good, 1977; Tabor, 1977). The adsorption theory is the most widely accepted theory of adhesion and has been studied in depth by both Kinloch and Huntsberger (Kinloch, 1980; Huntsberger, 1966; Huntsberger, 1967).

1.4.1.2.C. The wetting theory

The ability of bioadhesive polymers or mucus to spread and develop intimate contact with each other is an important factor for bond formation. The wetting theory, which has been predominantly applied to liquid adhesives, uses interfacial tensions to predict spreading and, in turn, adhesion (Peppas and Buri, 1985).
1.4.1.2.D. The diffusion theory

The interpenetration and entanglement of bioadhesive polymer chains and mucous chains produce semi permanent adhesive bonds is supported by this theory (Figure 1.2). The bond strength increases, with the degree of penetration of the polymer chains into the mucus layers. It has not been determined exactly how much penetration is required for effective bioadhesive bond, but it is believed to be in the range of 0.2 - 0.5 μm. It is possible to estimate the penetration depth (l) with the following formula:

\[ l = \sqrt{tD_b} \]

Where 't' is the time of contact and \( D_b \) is the diffusion coefficient of the bioadhesive material in mucus.

![Figure 1.2. Mechanical bonding through interpenetration of bioadhesive and mucus polymer chains.](image)

1.4.1.2.E. The fracture theory

This theory analyzes the forces required to separate two surfaces after adhesion. The maximum tensile stress \( (S_m) \) produced during detachment can be determined by dividing the maximum force of detachment, \( F_m \), by the total surface area, \( A_m \), involved in the adhesive interactions. The equation is:

\[ S_m = \frac{F_m}{A_m} \]

In a uniform single component system, fracture strength \( (S_f) \) which is equal to the maximum stress of detachment \( (S_m) \), is proportional to the fracture energy \( (g_f) \), young’s Modulus of Elasticity \( (E) \) and the critical crack length \( (C) \) of the fracture site as described by the following relationship below:
Fracture energy \((g_c)\) can be obtained from the sum of the irreversible work of adhesion, \(w_r\) (is energy required to produce new fracture surfaces), the reversible work of adhesion \((w_i)\), where both values are expressed per unit area of the fracture surface \((A_f)\), as below:

\[
g_c = w_r + w_i
\]

The elastic modulus of the system \((E)\) is related to stress \((\sigma)\) and strain \((\varepsilon)\) through Hook’s law:

\[
E = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta l/l_0} = \frac{F}{A_0} \cdot \frac{\Delta l}{l_0}
\]

Here, \(\varepsilon = \frac{\text{changing force (F)/Area (A)}}{\text{Original thickness (l_o)}}\)

### 1.4.2. Factors Affecting Mucoadhesion

The various factors affecting mucoadhesion are enlisted below:

(I) **Physical factors** (Chowdary and Srinivas, 2000):

(a) **Molecular weight**: Increased molecular weight of the bioadhesive polymer increases the bioadhesion.

(b) **Flexibility of polymer chains**: As the polymer chains will be more flexible more will be they able to penetrate the mucous layer and starts entanglement.

(c) **Spatial conformation**: Spatial conformation of a molecule is also important. Despite a high molecular weight of 19,500,000 for dextrans. They have adhesive strength similar to that of polyethyleneglycol, which has a molecular weight of 200,000. The helical conformation of electrons may shield many adhesively active groups, primarily responsible for adhesion unlike PEG polymers that have a linear conformation.
(d) Swelling: As the polymers start swelling, so, more will be the intimate contact of the polymers with the mucous layer as the polymer chains will more easily penetrate the mucous layer and thus entangle with the mucin chains.

(II) Environmental factors (Kamath and Park, 1995):

(a) pH of polymer-substrate interface: The adhesive strength increases as pH of the polymer substrate interface increases.

(b) Initial contact time: This factor between the mucoadhesive polymer and the mucus layer determines the extent of swelling and interpenetration of polymer chains. In general the mucoadhesive strength increases as the initial contact time increases. Although longer initial contact time may improve mucoadhesive strength and reproducibility of experiments, the optimum contact time, should be based on tissue viability.

(c) Initial pressure: If high pressure is applied for a sufficient long period of time polymers become mucoadhesive even though they do not have attractive interactions with the mucin. The intrinsic mucoadhesiveness can be determined by measuring the mucoadhesive strength at various initial pressures and extrapolating it to zero applied pressure.

(d) Selection of model substrate surface: The handling and treatment of biological substrate during the testing of mucoadhesives is an important factor, since, the physical and biological changes may occur in the mucus gels or tissues under experimental conditions.

(III) Physiological factors (Kamath and Park, 1995):

(a) Mucin turnover: This factor is important at least for two reasons as below:

(i) Mucin turnover is expected to limit the residence time of the mucoadhesives on the mucous layer. No matter, how high the mucoadhesive strength, mucoadhesives are detached from the surface due to mucin turnover.

(ii) Mucin turnover results in substantial amounts of soluble mucin molecules. These molecules interact with the mucoadhesives before they have a chance to interact
with the mucous layer. Moreover this factor may depend on several factors such as presence of food.

(b) Disease states: The physicochemical properties of the mucous changes during disease conditions such as common cold, gastric ulcer, ulcerative colitis, cystic fibrosis, bacterial and fungal infections of the female reproductive tract and inflammatory conditions of the eye. If the mucoadhesive microcapsules are to be used in these conditions then they are to be tested in these conditions only.

1.4.3. Preparation of Bioadhesive Microspheres

Bioadhesive microspheres can be prepared using any of the following techniques (Table 1.8).

1.4.4. Evaluation of Bioadhesive Microspheres

The best approach to evaluate bioadhesive microspheres is to evaluate the effectiveness of mucoadhesive polymer to prolong the residence time of drug at the site of absorption, thereby increasing absorption and bioavailability of the drug. The methods used to evaluate bioadhesive microspheres include the following.

1. Measurement of adhesive strength/ in vitro tests
   1.1. Tensile stress measurement
   1.1.1. Wilhelmy plate technique
   1.1.2. Novel electromagnetic force transducer (EMFT)
   1.2. Shear stress measurement
   1.3. Other tests to measure the adhesive strength
   1.3.1. Adhesion number
   1.3.2. Falling liquid film method
   1.3.3. Everted sac technique
   1.4. Novel rheological approach
2. Measurement of the residence time/ in vivo techniques
   2.1. GI transit using radio-opaque microspheres
   2.2. Gamma scintigraphy technique
3. Surface characterization of the bioadhesive microspheres
Table 1.8. Preparation of bioadhesive microspheres

<table>
<thead>
<tr>
<th>Process used</th>
<th>Particle size (μm)</th>
<th>Polymers</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent evaporation</td>
<td>1–100</td>
<td>Relatively stable polymers, e.g. polyesters, polystyrene</td>
<td>Labile polymers may degrade during the fabrication process.</td>
</tr>
<tr>
<td>Hot melt microencapsulation</td>
<td>1–1000</td>
<td>Water labile polymers, e.g. polyanhydrides, polyesters; with a molecular weight of 1000–50000</td>
<td>Smooth and dense external surfaces of the microspheres</td>
</tr>
<tr>
<td>Solvent removal</td>
<td>1–300</td>
<td>High melting point polymers especially polyanhydrides</td>
<td>Avoids use of water, only organic solvents are used</td>
</tr>
<tr>
<td>Spray drying</td>
<td>1–10</td>
<td>-</td>
<td>Primarily for microspheres used for intestinal imaging</td>
</tr>
<tr>
<td>Ionic gelation and size extrusion</td>
<td>1–300</td>
<td>Chitosan, CMC, alginate</td>
<td>Used for encapsulation of live cells</td>
</tr>
<tr>
<td>Phase inversion</td>
<td>0.5–5.0</td>
<td>Polyanhydrides</td>
<td>Involves low polymer loss and low drug loss during fabrication process</td>
</tr>
</tbody>
</table>
1.4.5. Pharmaceutical Applications

Bioadhesive microspheres have been extensively studied for a number of applications (Figure 1.3). Majority of these can be understood by classifying these applications on the basis of route of administration.

![Diagram of bioadhesive microspheres in drug delivery.](image)

Figure 1.3. Applications of bioadhesive microspheres in drug delivery.
1.5. SELECTION OF DRUGS, POLYMERS AND EXCIPIENTS

Development of an ideal controlled or sustained release formulation with desired characteristics in terms of safety, efficacy, patient compliance, aesthetics, acceptability to regulatory authorities, and cost requires a careful and meaningful selection of the polymer and other excipients.

1.5.1. Selection of Polymers and Excipients

The choice of an excipient for a particular formulation is governed by various critical parameters spanning from their origin to their regulatory status. Some of the important parameters can be listed as:

- Origin, source, and availability
- Functional category
- Quality and purity
- Impurity levels and extent of characterization
- Batch-to-batch consistency
- Stability of raw material and in the formulation
- Compatibility with the active ingredient and packaging material
- History of prior human use
- Safety and toxicological issues
- Cost considerations
- Biological activity(s), if any
- Regulatory and compendial status
- Patent status

The most common approach in designing controlled or sustained drug delivery systems is the use of polymers. Ideally, the polymers used in sustained drug delivery systems should possess the following characteristics:

1. Polymer should be sufficiently retardant i.e. it should be able to retard the release of the drug from the dosage form for sufficient period of time.
2. The polymer should be non-irritant and non-toxic.
3. The polymer should not bind the drug irreversibly i.e. it should allow the release of the drug molecule when in contact with biological fluids or buffer of similar composition.
4. The polymer should itself be stable on storage and during the shelf-life of the device or formulation.

5. The polymer should allow incorporation of large amounts of active ingredients without excessive deterioration of its mechanical properties.

6. It should not interact chemically with drugs or active constituents.

7. It should be commercially available and economically produced.

Polymers which are used widely in the controlled or sustained release drug delivery system are listed below:

1. Gums and mucilages e.g. xanthan gum, guar gum, sodium alginate, gellan gum, tragacanth, and pectin.

2. Cellulose derivatives e.g. ethylcellulose, methylcellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropylmethylcellulose (HPMC), hydroxyethyl cellulose (HEC) and sodium carboxy-methylcellulose (CMC).

3. Polyvinyl derivatives e.g. polyvinylalcohol, polyvinylacetate, polyvinylchloride.

4. Acrylates e.g. polyacrylamide, pHEMA, ethyl acrylate, poly acrylic acid salts and methacrylates.

5. Miscellaneous e.g. hyaluronic acid, carageenan, agrose, chitosan, PLGA, and starch acetate.

Based on the above mentioned criteria, the following polymers were selected for the present investigation:

- Sodium alginate
- Eudragit RSPO
- Talc
- Ispaghula husk

1.5.1.1. SODIUM ALGINATE

Alginic acid and its salts are abundantly present in brown algae (pheophyta) of the genera *Macrocystis, Laminaria, Ascophyllum, Alario, Ecklonia, Eisenia, Nercocystis, Sargassum, Cystoseira*, and *Fucus*.

Chemically alginates are linear, unbranched polysaccharide composed of monomers of β-D- mannuronic acid (M) and it’s C-5 epimer α-D- guluronic acid (G)
residues joined together by (1→4) glycoside linkages (Figure 1.4). These homopolymeric regions of β-D- mannanuronic acid blocks and α-L-guluronic acid blocks are interdispersed with regions of alternating structure (β-D-mannuronic acid –α-L-guluronic acid blocks). The composition and extent of the sequences and the molecular weight determine the physical properties of the alginates. The molecular variability is dependent on the organism and tissue from which the alginates are isolated (Currie, 1982).

Figure 1.4. Structure of polymeric blocks of alginic acid. (GG) guluronic sequence, (MM) mannanuronic sequence. (Khotimchenko, 2001).

1.5.1.1.1. Properties of alginates

1.5.1.1.1.A. Solubility

Sodium alginate is slowly soluble in cold water, forming viscous, colloidal solution. It is insoluble in alcohol and hydroalcoholic solutions in which alcohol content is greater than 30% by weight. It is also insoluble in other organic solvents like, chloroform, ether and in acids where pH of the resulting solution falls below 3.0. A 1% solution in distilled water has a pH of approximately 7.2. Calcium alginate, is however, practically insoluble in water and organic solvents but soluble in sodium citrate (Shilpa, 2003).

1.5.1.1.1.B. Viscosity

Various grades of sodium alginates are available, yielding aqueous solutions of varying viscosity within a range of 20-400 centipoises (0.02-0.4 PaS) in 1% solution at 20 °C. Due to distribution of chain lengths, alginate solutions are not clearly Newtonian and behave
as pseudoplastic fluid. When dissolved in pure water, their reduced viscosity is expected to increase very rapidly with dilution. In the presence of supporting electrolyte rheological behavior of polyelectrolyte solution is known to depend on the ionic structure of the aqueous solvent, e.g. increasing the concentration of a strong electrolyte such as NaCl in the alginate solution up to 100 mM was shown to reduce the solution viscosity due to the change in polymer conformation (Shilpa, 2003).

1.5.1.1. Chemical stability and degradation

Degradation of a Ca\(^{2+}\) cross-linked alginate gel can occur by removal of the Ca\(^{2+}\) ions. This can be accomplished by the use of a chelating agent such as ethylene glycol-bis (b-amino ethyl ether)-N, N', N'\text{-} tetra acetic acid (EGTA), lactate, citrate and phosphate or by a high concentration of ions such as Na\(^+\) or Mg\(^{2+}\). As Ca\(^{2+}\) ions are removed, the cross-linking in the gel decreases and the gels are destabilized. This can lead to leakage of entrapped material and solubilization of the high molecular weight alginate polymers. Alginate gels will also degrade and precipitate in a 0.1 M phosphate buffer solution and will completely dissolve in 0.1 M sodium citrate at pH 7.8. If Ca\(^{2+}\) is used in the cross-linking solution and phosphate buffer is used as the dissolution medium, the dissolution medium will turn turbid due to the Ca\(^{2+}\) dissociation from the polymer network and forming calcium phosphate precipitate. This phenomenon is more evident when a high guluronic content alginate is used. Low α-L-guluronic acid content alginate and lower molecular weight alginate are known to release encapsulated proteins at a much faster rate. Degradation of the gel can be prevented by storing the gel beads in a medium that contains free Ca\(^{2+}\) ions and to keep the Na\(^+\) : Ca\(^{2+}\) ratio less than 25:1 for high α-L-guluronic acid alginates and 3:1 for low α-L-guluronic acid alginates (Gombotz, 1998).

Alginate have been reported to undergo proton-catalyzed hydrolysis, which is dependent on time, pH, and temperature. A cross-linked alginate matrix delivery system when exposed to low pH can therefore undergo a reduction in alginate molecular weight, which results in faster degradation and release of a molecule when the gel is re-equilibrated in a neutral pH solution. Ability of alginate to form two types of gel depend on pH, i.e. an acid gel and an ionotropic gel, gives the polymer unique properties compared to neutral macromolecules (Tonnesen, 2002).
I.5.1.1.D. Sterilization

Filtration is the simplest and least detrimental means of aseptization but mostly alginate solutions are sterilized by autoclaving rather than filtration. It is reported that some decrease in viscosity occurs following sterilization by autoclaving, as the thermal process randomly cleaves alginate chains. γ-radiation and ethylene oxide have also been used to sterilize alginate solutions (Shilpa, 2003).

I.5.1.1.E. Bioadhesion

Alginate possesses a bioadhesive property, which could serve as a potential advantage in mucosal drug delivery. Alginate with its carboxyl end groups is classified as anionic mucoadhesive polymer, and studies have shown that alginate has the highest mucoadhesive strength compared with polymers such as polystyrene, chitosan, CMC, and poly (lactic acid), because it has been reported that polyanion polymers are more effective bioadhesive than polycation polymers or non-ionic polymers. This bioadhesive property of alginate serves as a potential advantage in mucosal drug delivery such as to the gastrointestinal tract and nasopharynx. These mucoadhesive drug delivery systems work by increasing the drug residence time at the site of activity or resorption and hence aid in it’s utility as a potential delivery vehicle for drugs to mucosal tissues. It also improves the overall drug effectiveness and bioavailability (Gaserod, 1998).

I.5.1.1.F. Gel properties

This polysaccharide has many anionic or cationic groups in the structure; therefore, it exhibits a unique physical property by electrostatic interaction. A property of aqueous solutions of alginate, which has been widely exploited for fabrication of vehicles for sustained delivery of bioactive molecules, is their ability to form firm gels on addition of divalent and trivalent metal ions such as bivalent alkaline earth metals (Ca++, Sr++, and Ba++) or trivalent Fe+++ and Al+++ ions. This is a result of ionic interaction and intramolecular bonding between the carboxylic acid groups located on the polymer backbone and the cations that are present (Katchalsky, 1961).

The regions of guluronate monomers in one alginate molecule can be linked to a similar region in another molecule by means of calcium or other divalent cations. In the presence of divalent calcium ions, the calcium is ionically substituted at the carboxylic site. A second alginate strand can also connect at the calcium ion, forming a link in which the Ca++
ion attaches two alginate strands together (Shilpa, 2003). The result is a chain of calcium-linked alginate strands that form solid gel. The divalent calcium cation fits into electronegative cavities like eggs in an egg-box; from this similitude arises the term ‘Egg Box’ model (Grant, 1983). This binds the alginate polymer together by forming junction zones, thus leading to gelation of solution (Mirghani, 2000). The cross-linking sites that occur when a polyvalent cation causes interpolysaccharide binding are called junction zones. The junction zone is an alignment of helices with two anhydroguluronic acid units per turn, the helices being held together by chelate bound Ca^{++}, which looks like the eggs in the pocket of an egg carton. As evident from Figure 1.5 (Al-Musa, 1999), only half of the carboxylate groups engage in chelate binding of calcium if the egg box is a dimerization of molecules. The rest of the Ca^{++} is ordinary bound. A multimeric junction zone is also possible with this model. The interpretation is that the dimeric junction zone binds the Ca^{++} strongly, which is inside the egg box, whereas the Ca^{++} outside the egg box is less strongly bound. Furthermore, it may be interpreted that the multimeric junction zones are less stable than dimeric zones. Ca^{++} and Ba^{++} bonding to alginate is expected to occur in a planer two-dimensional manner; on the other hand, trivalent aluminium cation is expected to form a three-dimensional valent bonding structure with alginate.

Figure 1.5. Schematic representation of the “egg-box” model of alginate gel. (a, b) Binding zones between polymeric alginate molecules; (c) an elementary cell of the binding zone. The dotted line indicates hydrogen bonds between the oxygen atoms of the pyranosic cycles and the metallic ion; the dashed line indicates ionic bonds between carboxyl groups and the metallic ion.
1.5.1.1.2. Useful properties of alginate as matrix for controlled drug delivery

Alginites have been widely used as tablet disintegrant, binding agent, viscosity-modifying agent, as a stabilizer in disperse system in the production of suspension and emulsion and also as thickening agent in pharmaceutical industries. The most important advantage of using alginate as a matrix for controlled release (CR) formulations is its biodegradability, because it is degraded and is absorbed by the body during and/or after drug release without any toxic effects. This allows bypass of surgical removal of the device. Hence, it can be a suitable matrix for sustained release of various drugs. Furthermore, because drug delivery can be controlled primarily through properties of polymer devices, CR is possible for conventional low molecular weight drugs as well as macromolecular drugs including peptide hormones (e.g., insulin, growth hormone), polysaccharides (e.g., heparin), antibiotics, antigens, and enzymes (Shilpa, 2003). The release of drugs from alginate beads occurs mainly by diffusion through matrix and at certain pH due to erosion mechanism (Takka, 1998). Release of drugs can be controlled by coating of matrix beads with sodium alginate. Sodium alginate has also been evaluated as release-controlling diluents in CR capsules. Several drugs have been incorporated into alginate matrices in a variety of forms (e.g., beads, microspheres, films, and tablets), for CR therapies (Hosny, 1998; Fattah, 1998).

The following properties of alginates have enabled it to be used as a matrix for controlled drug delivery (Fattah, 1998).

- It is readily available and is relatively inexpensive.
- It contains ingredients that are accepted food additives.
- It is non-toxic when taken orally and also has a protective effect on mucous membranes of upper gastrointestinal tract.
- It is hemocompatible and does not accumulate in any organ of the human body.
- It is biodegradable so there is no need for surgical removal after the drug is exhausted.
- It can form hydrogels under mild conditions.
- It is water-soluble so it eliminates the use of noxious solvents during processing and hence stability, toxicological, and environmental problems associated with solvents can be minimized.
- It forms gel at room temperature and hence reduces chances of destroying activity of sensitive drugs at elevated temperatures.
Soluble sodium alginate cross-linked with a variety of cross-linking agents forms insoluble gel, which is used for prolong active drug delivery system.

Flow properties of drugs with needlelike crystals (e.g., Sulfadiazine) can be improved by incorporating in alginate beads. This method of agglomeration also avoids polymorphic transformations as agglomerates are formed from drug dispersions.

Beads formed are mechanically strong so they could be coated with enteric polymers to prepare enteric drug delivery systems.

1.5.1.2. EUDRAGIT RSPO (Chang et al., 2005)

1.5.1.2.1. Chemical name

Poly (ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) 1: 2: 0.1

1.5.1.2.2. CAS number- 33434-24-1

1.5.1.2.3. Structural formula

![Figure 1.6. Structural formula of Eudragit RSPO.](image)

\[ R^1 = H, \text{CH}_3, \quad R^2 = \text{CH}_3, \text{C}_2\text{H}_5, \quad R^3 = \text{CH}_3, \quad R^4 = \text{CH}_2\text{CH}_2\text{N(CH}_3)_3\text{Cl}^2 \]

1.5.1.2.4. Molecular weight

Typically, the molecular weight of the polymer is 100000.

1.5.1.2.5. Functional category

Film former, tablet binder, tablet diluent.
1.5.1.2.6. Description

Eudragit RSPO is synthetic cationic and anionic polymers of Poly (ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) in 1:2:0.1 ratio. It is fine, white powder with a slight amine-like odor. They contain about 97% of dry polymer. The recommended solvents or diluents for Eudragit RSPO may include acetone and alcohols. It shows low permeability characteristics. It is soluble in acetone and alcohols, dichloromethane, ethyl acetate; and insoluble in 1 N HCl, 1 N NaOH, petroleum ether and water.

Alkali value: 12.1–18.3
Density (bulk): 0.390 g/cm³
Density (tapped): 0.424 g/cm³
Density (true): 0.816–0.836 g/cm³

1.5.1.2.7. Stability and storage conditions

It is stable at temperatures less than 30 °C. Above this temperature, powders tend to form clumps, although this does not affect the quality of the substance and the clumps can readily be broken up. Dry powder of Eudragit RSPO is stable for at least 3 years if stored in a tightly closed container at less than 30 °C.

1.5.1.2.8. Incompatibilities

Eudragit RSPO, due to its dry powdered form is generally compatible with most of the existing drugs.

1.5.1.2.9. Safety

Eudragit RSPO is widely used as film-coating materials in oral pharmaceutical formulations. It is generally regarded as nontoxic and nonirritant materials. A daily intake of 2 mg/kg body-weight of Eudragit RSPO (equivalent to approximately 150 mg for an average adult) may be regarded as essentially safe in humans.

1.5.1.2.10. Handling precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection, gloves, and a dust mask or respirator are recommended. Polymethacrylates should be handled in well-ventilated environment and measures should be taken to prevent dust formation.
1.5.1.2.11. Regulatory status

Eudragit RSPO is included in the FDA Inactive Ingredients Guide (oral capsules and tablets). It is included in nonparenteral medicines licensed in the UK. It is also included in the Canadian List of Acceptable Non-medicinal Ingredients.

1.5.1.2.12. Applications in pharmaceutical formulation or technology

Eudragit RSPO is primarily used in oral capsule and tablet formulations as film-coating agents. It is used to form water-insoluble film coats for sustained-release products. Eudragit RSPO films are less permeable. It is also used as binders in both aqueous and organic wet-granulation processes. Larger quantities (5–20 %) of dry polymer are used to control the release of an active substance from a tablet matrix. Solid polymers may be used in direct-compression processes in quantities of 10–50 %.

1.5.1.3. ETHYLCELLULOSE (Dahl, 2005)

Ethylcellulose, an ethyl ether of cellulose, is a long-chain polymer of β-anhydroglucose units joined together by acetal linkages.

1.5.1.3.1. Synonyms: Ethocel, Surelease

1.5.1.3.2. Chemical name and CAS registry number: Cellulose ethyl ether [9004-57-3]

1.5.1.3.3. Structural formula

![Figure 1.7. Structural formula of ethylcellulose.](image)

Where, \( n \) can vary to provide a wide variety of molecular weights.
1.5.1.3.4. Functional category

Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity increasing agent.

1.5.1.3.5. Description

Ethylcellulose is a tasteless, free-flowing, and white to light tan-colored powder.

Typical Properties

Density (bulk): 0.4 g/cm³

Glass transition temperature: 129–133 °C

Specific gravity: 1.12–1.15 g/cm³

Moisture content

Ethylcellulose absorbs very little water from humid air or during immersion, and that small amount evaporates readily.

1.5.1.3.6. Solubility

Ethylcellulose is practically insoluble in glycerin, propylene glycol, and water. Ethylcellulose that contains less than 46.5 % of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95 %). Ethylcellulose that contains not less than 46.5 % of ethoxyl groups is freely soluble in chloroform, ethanol (95 %), ethyl acetate, methanol, and toluene.

1.5.1.3.7. Viscosity

The viscosity of ethylcellulose is measured typically at 25 °C using 5 % w/v ethylcellulose dissolved in a solvent blend of 80 % toluene: 20 % ethanol (w/w). Grades of ethylcellulose with various viscosities are commercially available. They may be used to produce 5 % w/v solutions in organic solvent blends with viscosities nominally ranging from 7 to 100 mPa s (7–100 cP). Specific ethylcellulose grades, or blends of different grades, may be used to obtain solutions of a desired viscosity. Solutions of higher viscosity tend to be composed of longer polymer chains and produce strong and durable films.

The viscosity of an ethylcellulose solution increases with an increase in ethylcellulose concentration; e.g. the viscosity of a 5 % w/v solution is 4 mPa s (4 cP) and of a 25 % w/v solution of the same ethylcellulose grade is 850 mPa s (850 cP). Solutions with a lower viscosity may be obtained by incorporating a higher percentage (30–40 %) of a low-molecular-weight aliphatic alcohol such as ethanol, butanol, propan-2-ol, or n-butanol with
toluene. The viscosity of such solutions depends almost entirely on the alcohol content and is independent of toluene. In addition, nonpharmaceutical grades of ethylcellulose that differ in their ethoxyl content and degree of polymerization are available.

1.5.1.3.8. Stability and storage conditions

Ethylcellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters.

Ethylcellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230–340 nm range.

Ethylcellulose should be stored at a temperature not exceeding 32 °C in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

1.5.1.3.9. Incompatibilities

Incompatible with paraffin wax and microcrystalline wax.

1.5.1.3.10. Applications in pharmaceutical formulation or technology

Ethylcellulose is widely used in oral and topical pharmaceutical formulations. The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation. Modified-release tablet formulations may also be produced using ethylcellulose as a matrix former. Ethylcellulose, dissolved in an organic solvent or solvent mixture, can be used to produce water-insoluble films. Higher-viscosity ethylcellulose grades tend to produce stronger and more durable films. An aqueous polymer dispersion (or latex) of ethylcellulose may also be used to produce ethylcellulose films without the need for organic solvents.

Drug release through ethylcellulose-coated dosage forms can be controlled by diffusion through the film coating. This can be a slow process unless a large surface area (e.g. pellets or granules compared with tablets) is utilized. In those instances, aqueous ethylcellulose dispersions are generally used to coat granules or pellets. Ethylcellulose-coated beads and granules have also demonstrated the ability to absorb pressure and hence protect the coating from fracture during compression.
High-viscosity grades of ethylcellulose are used in drug microencapsulation. Release of a drug from an ethylcellulose microcapsule is a function of the microcapsule wall thickness and surface area.

In tablet formulations, ethylcellulose may additionally be employed as a binder, the ethylcellulose being blended dry or wet-granulated with a solvent such as ethanol (95%). Ethylcellulose produces hard tablets with low friability, although they may demonstrate poor dissolution.

Ethylcellulose has also been used as an agent for delivering therapeutic agents from oral (e.g., dental) appliances. In topical formulations, ethylcellulose is used as a thickening agent in creams, lotions, or gels, provided an appropriate solvent is used. Ethylcellulose has been studied as a stabilizer for emulsions. Ethylcellulose is additionally used in cosmetics and food products.

1.5.1.4. TALC (Kibbe, 2005)
1.5.1.4.1. Synonyms

Hydrous magnesium calcium silicate, hydrous magnesium silicate, magnesium hydrogen metasilicate, powdered talc; purified French chalk, soapstone, steatite.

1.5.1.4.2. Chemical name and CAS registry number- Talc [14807-96-6]

1.5.1.4.3. Empirical formula and molecular weight

Talc is a purified, hydrated, magnesium silicate, approximating to the formula \( \text{Mg}_6(\text{Si}_2\text{O}_5)\text{H}_4 \). It may contain small, variable amounts of aluminum silicate and iron.

1.5.1.4.4. Functional category

Anticaking agent, glidant, tablet and capsule diluents, tablet and capsule lubricant.

1.5.1.4.5. Applications in pharmaceutical formulation or technology

Talc was once widely used in oral solid dosage formulations as a lubricant and diluent, although today it is less commonly used. However, it is widely used as a dissolution retardant in the development of controlled-release products. Talc is also used as a lubricant in tablet formulations; in a novel powder coating for extended-release pellets; and as an adsorbant.
In topical preparations, talc is used as a dusting powder, although it should not be used to dust surgical gloves. Talc is a natural material; it may therefore frequently contain microorganisms and should be sterilized when used as a dusting powder. Talc is additionally used to clarify liquids and is also used in cosmetics and food products, mainly for its lubricant properties.

1.5.1.4.6. Description

Talc is a very fine, white to grayish-white, odorless, impalpable, unctuous, and crystalline powder. It adheres readily to the skin and is soft to the touch and free from grittiness.

1.5.1.4.7. Typical properties

Acidity/alkalinity: pH = 7–10 for a 20 % w/v aqueous dispersion.

Hardness (Mohs): 1.0–1.5

Moisture content: Talc absorbs insignificant amounts of water at 25 °C and relative humidity up to about 90%.

Particle size distribution: Varies with the source and grade of material. Two typical grades are 99 % through a 74 μm (#200 mesh) or 99 % through a 44 μm (#325 mesh).

Refractive index: n^20/D 1.54–1.59

Solubility: Talc is practically insoluble in dilute acids and alkalis, organic solvents, and water.

Specific gravity: 2.7–2.8

Specific surface area: 2.41–2.42 m^2/g

1.5.1.4.8. Stability and storage conditions

Talc is a stable material and may be sterilized by heating at 160 °C for not less than 1 h. It may also be sterilized by exposure to ethylene oxide or gamma irradiation. Talc should be stored in a well-closed container in a cool, dry place.

1.5.1.4.9. Incompatibilities

Incompatible with quaternary ammonium compounds.

1.5.1.4.10. Safety

Talc is used mainly in tablet and capsule formulations. Talc is not absorbed systemically following oral ingestion and is therefore regarded as an essentially nontoxic
material. However, intranasal or intravenous abuse of products containing talc can cause granulomas in body tissues, particularly the lungs. Contamination of wounds or body cavities with talc may also cause granulomas; therefore, it should not be used to dust surgical gloves. Inhalation of talc causes irritation and may cause severe respiratory distress in infants. Although talc has been extensively investigated for its carcinogenic potential, and it has been suggested that there is an increased risk of ovarian cancer in women using talc, the evidence is inconclusive. However, talc contaminated with asbestos has been proved to be carcinogenic in humans, and asbestos-free grades should therefore be used in pharmaceutical products. Also, long-term toxic effects of talc contaminated with large quantities of hexachlorophene caused serious irreversible neurotoxicity in infants accidentally exposed to the substance.

1.5.1.4.11. Handling precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Talc is irritant if inhaled and prolonged excessive exposure may cause pneumoconiosis.

1.5.1.4.12. Regulatory status

Talc is accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (buccal tablets; oral capsules and tablets; rectal and topical preparations). It is included in nonparenteral medicines licensed in the UK. It is also included in the Canadian List of Acceptable Non-medicinal Ingredients.

1.5.1.5. ISPAGHULA HUSK

Psyllium is the common name used for several members of the plant genus *plantago*. Psyllium and ispaghula husk (similar uses differ in doses) terms are interchangeably used, psyllium is derived from the dried, ripe seeds of *Plantago psyllium* (synonym for *Plantago afra* L.) and from *Plantago indica* L., but ispaghula husk is derived from the ripe seeds of *Plantago ovata* Forsskaol (synonym for *Plantago isphagula* Roxburgh) (Blumenthal et al., 2000). The seeds of psyllium are used commercially for the production of mucilage. The mucilage obtained from the seed coat by mechanical milling/grinding of the outer layer of the seeds. It is a white fibrous hydrophilic material and forms the clear colourless mucilaginous gel by absorbing water. The gel nature and composition of the polysaccharides extracted from the seeds of the *P. ovata* has been reported in literature (Kennedy et al., 1979; Sandhu et al., 1981; Laidlaw and Purcival, 1950). Fischer et al. have studied the
physiologically active, gel-forming fraction of the alkali-extractable polysaccharides of *P. ovata Forsk* seed husk (psyllium seed) and some derived partial hydrolysis products by compositional and methylation analysis and NMR spectroscopy. Chemical and physical studies of the active fraction of psyllium mucilage shows that it has arabinose 22.6 %, xylose 74.6 %, molar basis; only traces of other sugars. With about 35 % of non-reducing terminal residues, the polysaccharide is highly branched. The data are compatible with a structure consisting of a densely substituted main chain of β-(1→4)-linked d-xylopyranosyl residues, some carrying single xylopyranosyl side chains at position 2, others bearing, at position 3, trisaccharide branches having the sequence 1-Araβ-(1→3)-d-Xylp-β-(1→3)-1-Araβ. The presence of this sequence is supported by methylation and NMR data, and by the isolation of the disaccharide 3-O-β-d-xylopyranosyl-arabinose as a product of partial acid hydrolysis of the polysaccharide (Fischer et al., 2004). Psyllium has been reported as a medicinally active natural polysaccharide. It has been used for the treatment of constipation (Bouchoucha et al., 2004; Ramkumar and Rao, 2005), diarrhea (Washington et al., 1998), inflammation bowel diseases-ulcerative colitis (Fernandez-Banares et al., 1999), obesity in children and adolescents (Pittler and Ernst, 2004), high cholesterol (Rodriguez-Moran et al., 1998; Moreyra et al., 2005; Romero et al., 2002; Anderson et al., 1995, 1999, 2000a,b) and diabetes (Anderson et al., 1999; Florholmen et al., 1982; Fagerberg, 1982; Gupta et al., 1994; Fukagawa et al., 1990; Pastors et al., 1991). Psyllium polysaccharides are also used in the treatment of constipation, diarrhea, irritable bowel syndrome, inflammatory bowel disease-ulcerative colitis, colon cancer, diabetes and hypercholesterolemia.

A number of drug delivery devices have been proposed to deliver the drug for efficient therapy (Chourasia and Jain, 2003). Among them, hydrogels, specially based on polysaccharides, have attracted considerable attention as an excellent candidate for controlled release devices or targetable devices of the therapeutic agents (Chourasia and Jain, 2004). The release rate of drugs from hydrogels was primarily determined by the swelling extent, which further enhanced by addition of enzyme in the buffer solutions (Chiu et al., 1999) whereas swelling of polymeric network was depend on composition of copolymer and pH of the surrounding medium (El-Hag Ali Said, 2005). Modification of the psyllium to develop the hydrogels is not much reported in the literature. Singh and coworkers have modified the psyllium to prepare the hydrogels for the special applications (Singh et al., 2006).
1.5.1.5.1. Safety aspects of psyllium

In order to find the safety and tolerability aspects of ispaghula husk various studies have been carried out. In one study the nutritional, biochemical and haematological effects of ispaghula has been studied. It was observed that a daily dose of 10.5 g of ispaghula was well tolerated and the majority of adverse events recorded were minor, of short duration and either unrelated or possibly related to the study treatment. The results from the study suggested that ispaghula husk could be used with confidence for the long-term treatment of mild-to-moderate hypercholesterolemia (Oliver, 2000). US Food and Drug Administration recently authorized the use of health claims on food products containing soluble fiber from psyllium that state that they are associated with a decreased risk of coronary heart disease (Anderson et al., 2000b). The addition of psyllium to a traditional diet for persons with diabetes is safe, it is well tolerated, and improves glycemic and lipid control in men with type 2 diabetes and hypercholesterolemia (Anderson et al., 1999). Ramkumar and co-workers have undertaken a systematic review of the efficacy and safety of traditional medical therapies for chronic constipation with the intention to make evidence-based recommendations. In their study they have found good evidence to support the use of tegaserod, lactulose, and psyllium for constipation (Ramkumar and Rao, 2005).
1.6. DRUG PROFILES

1.6.1. ISONIAZID

1.6.1.1. General

Generic Name: Isoniazid
Molecular Weight: 137.14
Category: Antitubercular

Molecular Formula: C₆H₇N₃O

Chemical Name: 4-pyridinecarboxylic acid, hydrazide

Dose: 300 mg daily or up to 1 g twice daily

1.6.1.2. Pharmacopoeial Specific action

Description: Colorless crystals or white crystalline powder; odorless.

Solubility: Freely soluble in water; sparingly soluble in ethanol (95 %); slightly soluble in chloroform; very slight soluble in ether.

Storage: Store in well closed, light resistant containers.

Standards: Isoniazid contains not less than 98.0 % and not more than 101.0 % of C₆H₇N₃O, calculated with reference to the dried substance.

Melting point: 170-173 °C

pH: Between 6.0 to 8.0, determined in a 5 % w/v solution.

Loss on drying: Not more than 0.5 %, determined on 1 g by drying in an oven at 105 °C.
1.6.1.3. Pharmacology of Isoniazid

1.6.1.3.1. Mechanism of action

It inhibits mycolic acid synthesis in susceptible bacteria resulting in loss of acid fastness and disruption of bacterial cell wall. Isoniazid is a highly specific agent and is active against organism of the genus Mycobacterium namely, *M. tuberculosis, M. bovis*. *In vitro*, the minimum inhibitory concentration (MIC) for most susceptible mycobacterium is 0.02-0.2 μg/mL in Lowenstein-Jensen media.

1.6.1.3.2. Resistance

Natural and acquired resistance to isoniazid have been demonstrated *in vitro* and *in vivo* in strains of *M. tuberculosis*. The mechanism of resistance may be related to failure of the drug to concentrate or to be taken up by the resistant bacteria.

1.6.1.3.3. Pharmacokinetics

Absorption

Isoniazid is readily absorbed from the gastrointestinal tract and from I.M. injection sites. When administered orally with food, the extent of absorption and peak plasma concentration of the drug may be reduced (Table 1.9).

Distribution

Isoniazid is distributed into all the body tissue and fluids. CSF concentration of the drug is reported to be 90-100% of the concurrent plasma concentration (Table 1.9).

Elimination and extraction

Isoniazid is extensively metabolized in liver, mainly by acetylation and dehydrazination. Metabolites of the drug include acetylisoniazid, isonicotinic acid, monoacetylhydrazin, diacetylhydrazin and isonicotinyl glycine. Acetylation of acetylisoniazid results in the formation of monoacetylhydrazine which has been shown to be a potent hepatotoxin in animals. Thus, although rapid inactivators form more monoacetylhydrazine, they also inactivate it more rapidly.

Kidney: In adult with normal renal function, approximately 50 to 70% of a 5 mg/kg oral dose is extracted in urine within 24 h as unchanged drug and as metabolites. The percentage of the different compounds extracted varies with the acetylator phenotype.
Chapter 1

Introduction

<table>
<thead>
<tr>
<th>Acetylators</th>
<th>Acetyl isoniazid and metabolites (%)</th>
<th>Free isoniazid and hydrazine conjugates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow acetylators</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td>Fast acetylators</td>
<td>94</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1.9. List of the various pharmacokinetic parameters for isoniazid

<table>
<thead>
<tr>
<th>Pharmacokinetic data</th>
<th>Rapid acetylators</th>
<th>Slow acetylators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral bioavailability (%)</td>
<td>Completely absorbed; food decrease the oral bioavailability.</td>
<td></td>
</tr>
<tr>
<td>Urine excretion (%)</td>
<td>7±2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>29±5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma binding (%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Clearance (mL/min.kg)</td>
<td>7.4±2&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.7±1.1&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume of distribution (L/kg)</td>
<td>0.67±0.15; ← Aged, RD&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;90&lt;/sub&gt; (h)</td>
<td>1.1±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1±1.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.1±0.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.1±0.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</td>
<td>5.4±2.0 µg/mL&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.1±1.9 µg/mL&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>It is usually stated that isoniazid is completely absorbed; however, good estimates of possible loss due to first pass metabolism are not available. Absorption is decreased by food. <sup>b</sup>After oral absorption, assay includes unchanged drugs and acid-labile hydrazones. Higher percentage has been noted after IV. administration, suggesting significant first-pass metabolism. <sup>c</sup>Metabolism by N-Acetyltrnasferase 2 (Polymorphic). <sup>d</sup>CL/F and V<sub>d</sub>/F are reported. <sup>e</sup>Decrease in CL<sub>NR</sub>/F as well CL<sub>NR</sub>. <sup>f</sup>Following a single 400 mg oral dose to healthy rapid and slow acetylators.

Abbreviations
C<sub>max</sub> = maximum plasma concentration, V<sub>d</sub> = volume of distribution, t<sub>max</sub> = time to maximum plasma concentration, V<sub>ss</sub> = volume of distribution at steady state, t<sub>90</sub> = elimination half life, CL = clearance (R = renal and NR = non-renal), F = fraction of drug available, RD = renal disease, AVH = acute viral hepatitis, Cirr = hepatic cirrhosis, Neo = neonate, HTH = hyperthyroid.
Breast milk: 0.75 to 2.3% of the dose is excreted into breast milk in 24 h. This corresponds to 6-20% of a usual therapeutic pediatric dose.

Other routes: Small amount of the drug are excreted in saliva, sputum and faeces.

1.6.1.3.4. Drug interactions

- Adverse nervous system effects of isoniazid, cycloserine and ethionamide may be additive therefore isoniazid should be used with caution in patients receiving cycloserin or ethionamide.
- Aminosalicylic acid appears to reduce the rate of acetylation of isoniazid but the effect is not clinically important.
- Isoniazid inhibits multiplication of BCG; therefore BCG vaccine may not be effective if administered during therapy with the drug.
- Initiation of isoniazid therapy (200 or 300 mg daily) in patients receiving carbamazepine results in increased serum concentration of the anticonvulsant and symptoms of carbamazepine toxicity, including ataxia, headache, vomiting, blurred vision, drowsiness and confusion.
- Isoniazid inhibit hepatic metabolism of phenytoin, resulting in increased plasma concentration of phenytoin and toxicity occurs mainly in slow acetylators and in patients receiving both isoniazid and aminosalicylic acid.
- Aluminium hydroxide gel decreases gastrointestinal absorption of isoniazid; isoniazid should be administered at least 1 h before the antacid.
- Coordination difficulties and psychotic episodes have been reported in patients receiving isoniazid and disulfiram concurrently due to alteration in metabolism.

1.6.1.3.5. Laboratory tests

Because there is a higher frequency of isoniazid associated hepatitis among certain patient groups, including age > 35 years daily user of alcohol, chronic liver disease, and women belonging to minority groups, particularly in post-partum period, transaminase measurements should be obtained prior to starting and monthly during preventive therapy, or more frequently as needed.
1.6.1.3.6. Therapeutic use

Isoniazid is used in conjunction with at least one other antitubercular agent to prevent the development and in the treatment of clinical tuberculosis.

1.6.1.3.7. Adverse effects

Nervous system effects

Peripheral neuritis, other adverse nervous system effects include seizures, toxic encephalopathy, muscle twitching, ataxia, stupor, euphoria, memory impairment, separation of ideas and reality, loss of self-control, dizziness and toxic psychosis.

Hepatic effects

Mild hepatic dysfunction, as evidenced by mild and transient increase in serum SGOT, SGPT and bilirubin concentrations, has occurred in approximately 10-20% of patients receiving isoniazid, usually during the first 4-6 months of the therapy.

Sensitivity reactions

Hypersensitivity reactions, including fever, skin eruptions (morbilliform, maculopapular, purpuric or exfoliative), lymphoadenopathy, vasculitis and rarely hypotension have occurred with isoniazid, usually 3-7 weeks following initiation of therapy.

Hematological effects

Adverse hematological effects, including agranulocytosis, eosinophilia, thrombocytopenia, methemoglobinemia and hemolytic, sideroblastic or aplastic anaemia have occurred in patients receiving isoniazid.

Other adverse effects

Other reported adverse effects of isoniazid include nausea, vomiting, epigastric distress, dryness of mouth, pyridoxine deficiency, pellagra, hyperglycemia, metabolic acidosis and urinary retention and gynaecomastia in males.

1.6.1.3.8. Precautions and contraindications

- Liver function test should be performed periodically in patients receiving isoniazid.
- Isoniazid should be used with precaution in daily user of alcohol and in patients with chronic liver disease or severe renal impairment.
Periodic ophthalmic examinations should be performed in patients who developed visual symptoms while receiving the drugs.

When isoniazid is used in patients who are malnourished or predisposed to neuropathy (e.g. diabetics, alcoholics), pyridoxine should generally be administered concomitantly.

Isoniazid is contraindicated in patients with acute liver disease or a history of previous isoniazid associated hepatic injury.

1.6.1.3.9. Carcinogenesis and mutagenesis

Isoniazid has been shown to induce pulmonary tumors in a number of strains of mice. Isoniazid has been found to be weakly mutagenic in strains TA100 and TA1535 of *Salmonella typhimurium* without metabolic activation.

1.6.1.3.10. Contraindications

Isoniazid is contraindicated in patients who develop severe hypersensitivity reactions, including drug induced hepatitis; previous isoniazid associated hepatic injury; severe adverse reactions to isoniazid such as drug fever, chills, arthritis; and acute liver disease of any etiology.

1.6.1.3.11. Overdose

**Signs and symptoms**

Nausea, vomiting, dizziness, slurring of speech, blurred vision and visual hallucinations, respiratory distress and CNS depression progressing rapidly from stupor to profound coma, are to be expected, along with severe, intractable seizures.

**Treatment**

Untreated or inadequately treated cases of gross isoniazid over dosage, 80 mg/kg to 150 mg/kg, can cause neurotoxicity, but good response has been reported in most patients brought under adequate treatment within the first few h after drug ingestion.

*Asymptomatic treatment:* Absorption of drug from the GIT may be decreased by giving activated charcoal. Gastric emptying should also be employed in the asymptomatic patient.

*Symptomatic treatment:* Ensure adequate ventilation, support cardiac output and protect the airway while treating seizures and attempting to limit adsorption.
General

Obtain blood samples for immediate determination of gases, electrolytes, BUN, glucose etc.

Dialysis

Both peritoneal and hemodialysis have been used in the management of isoniazid over dosage.

1.6.1.3.12. Dosage and administration

Isoniazid is usually given orally. It may be given by I.M. injection, only when orally not possible.

➢ Adults: 5 mg/kg up to 300 mg daily in a single dose; or 15 mg/kg up to 900 mg daily, two or three times per week.
➢ Children: 10-15 mg/kg up to 300 mg daily in a single dose; or 20-40 mg/kg up to 900 mg daily, two or three times per week.
1.6.2. RIFAMPICIN

1.6.2.1. General

**Generic Name:** Rifampicin, Rifampin  
**Molecular formula:** $\text{C}_{43}\text{H}_{58}\text{N}_{4}\text{O}_{12}$  
**Category:** Antitubercular

**Molecular weight:** 822.95

**Structural formula**

![Structural formula of rifampicin](image-url)


**Dose:** For an adult, 450 to 600 mg (about 10 mg/kg) daily preferably before breakfast. For a child, up to $20$ mg/kg daily to a maximum of $600$ mg.

1.6.2.2. Pharmacopoeial Specification

**Description:** Brick red to reddish brown, crystalline powder; practically odorless.

**Solubility:** Soluble in chloroform and in methanol; slightly soluble in acetone, in ethanol (95%), in ether and in water.

**Storage:** Store in well closed, light resistant containers in an atmosphere of nitrogen and in a cool place.

**Standards:** Rifampicin contains not less than 97.0 % and not more than 102.0 % of $\text{C}_{43}\text{H}_{58}\text{N}_{4}\text{O}_{12}$ calculated with reference to the dried substance.
Pka: 2

**pH:** Between 4.5 to 6.5, determined in a 1 % w/v suspension.

**Loss on drying:** Not more than 1 %, determined on 1 g by drying in an oven at 85 °C at a pressure not more than 0.7 kPa for 4 h.

1.6.2.3. Pharmacology of Rifampicin

1.6.2.3.1. Mechanism of action

Rifampicin has high activity against *M. tuberculosis* and *M. leprae*. It is also active against *Staphylococcus aureus*, coagulase negative *staphylocci*, *Listeria monocytogenes*, *Nisseria meningitides*, *Haemophylus influenzae*, *Legionella* spp., *brucella*, some strains of *E.coli*, *Proteus mirabilis*, anaerobic cocci, *Clostridium* spp., and bacteriodes. Rifampicin may be bacteriostatic or bactericidal depending on the concentration of drug attained at the site of action. The bactericidal actions are secondary to interfering with the synthesis of nucleic acid by inhibiting bacterial DNA-dependent RNA polymers at the β-subunit thus preventing initiation of RNA transcription, but not chain elongation.

1.6.2.3.2. Resistance

Resistance to rifampicin has been observed in *M.tuberculosis*, *M. kansasii* and in most bacteria susceptible to the drug. *In vitro*, resistance to rifampicin develops in a one-step process, probably as a result of modification of the β-subunit of RNA polymerase. Resistant strains develop rapidly if rifampicin is used alone in the treatment of clinical tuberculosis. Strains of *M. leprae* resistant to rifampicin have been reported rarely.

1.6.2.3.3. Pharmacokinetics

**Absorption**

Rifampicin is readily absorbed from the gastrointestinal tract (90 %). Peak plasma concentration occurs at 1.5 to 4 h after an oral dose (Table 1.10).

**Distribution**

Intravenous rifampicin has the same distribution as in oral route (Table 1.10).

**Metabolism**

Rifampicin is metabolized by the liver microsomal enzymes to its main and active metabolite deacetylrifampicin.
Table 1.10. Pharmacokinetic data for rifampicin

<table>
<thead>
<tr>
<th>Pharmacokinetic data</th>
<th>Reported values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral bioavailability (%)</td>
<td>Insufficient data&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary excretion (%)</td>
<td>7±3, ↑Neo</td>
</tr>
<tr>
<td>Plasma binding (%)</td>
<td>60-90</td>
</tr>
<tr>
<td>Clearance (mL/min.kg)</td>
<td>3.5±1.6&lt;sup&gt;d&lt;/sup&gt;, ↑Neo, ↔Aged, RD&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>0.97±0.36, ↔Aged, ↑Neo</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>3.5±0.8&lt;sup&gt;d&lt;/sup&gt;, ↑Hep, Cirr, Neo, RD&lt;sup&gt;e&lt;/sup&gt;, ↔Aged, Child</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1-3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>6.5±3.59 μg/mL&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Active desacetyl metabolite, <sup>b</sup>Some studies indicate complete absorption due to active metabolite, but first-pass metabolism is expected, <sup>c</sup>Not observed with 300mg doses, pronounced differences with 900 mg doses, <sup>d</sup>Half-life longer with high single doses. Shorter half-life and CL/F higher after repeated administration due to autoinduction of metabolism, <sup>e</sup>Following a 600 mg dose given daily for 15-18 days to patients with tuberculosis.

**Abbreviations**

C<sub>max</sub> = maximum plasma concentration, V<sub>d</sub> = volume of distribution, t<sub>max</sub> = time to maximum plasma concentration, t<sub>1/2</sub> = elimination half life, CL= clearance (R= renal and NR= non-renal), RD = renal disease, Cirr = hepatic cirrhosis, Hep = hepatitis.

**1.6.2.3.4. Drug interactions**

- Food lowers the peak blood level because of interference with absorption of rifampicin.
- Antacids containing aluminium hydroxide reduced the bioavailability of rifampicin.
- Para-amino salicylic acid granules may delay rifampicin absorption because bentonite present as a granule excipient, which leads to an inadequate serum level of rifampicin.
- Isoniazid and rifampicin interaction has led to hepatotoxicity. Alcohol intake with rifampicin increases the risk for hepatotoxicity.
- Rifampicin induces the microsomal enzymes of the liver and therefore accelerates metabolism of some drugs, e.g. beta blockers, calciferol, coumarins, cyclosporine,
dapson, diazepam, digitalis, hexobarbitone, ketoconazole, methadone, oral contraceptive pills, oral hypoglycemics, phenytoin, sulphasalazin, theophylline, some antiarrhythmics such as disopyramide, lorcainide, mexilitine, quinidin and verapamil.

- Rifampicin induces liver steroid metabolizing enzymes thus lowering the levels of glucosteroids and mineralocorticoids.
- When rifampicin and cyclosporine are taken, the serum levels of cyclosporine may be lowered.
- In the therapy of leprosy, rifampicin may induce dapsone metabolism, however, this is of minor significance in the clinical setting.
- The clinical condition of patients, who are on rifampicin and also taking digoxin for heart failure, may deteriorate because of falling digoxin levels.
- Patients on methadone maintenance for narcotic detoxification may develop narcotic withdrawal when methadone plasma levels decreased as a consequence of taking rifampicin at the same time.
- Rifampicin induce hepatic enzyme metabolism which can decrease metaprolol blood level, although this may be clinically insignificant.
- In patients who are receiving rifampicin and phenytoin together, there is an increase of clearance of phenytoin by two fold, significantly reducing the effect of the anticonvulsant drug.
- Modification of quinidine dose is necessary when this is used with rifampicin because of the risk of ventricular dysrrhythmias.
- When verapamil and rifampicin are taken together, rifampicin induces the liver enzymes which increase the metabolism of the calcium channel blocker leading to undetectable verapamil levels in blood.

1.6.2.3.5. Therapeutic use

- The primary indications for rifampicin are for treatment of tuberculosis (pulmonary as well as extrapulmonary) and for leprosy.
- It also useful for elimination of Neisseria meningococci in carriers and for gram positive and gram negative bacteria.
- It has some antilamydial activity and in vitro activity against some viruses (poxvirus and adenovirus) at high dose.
- It has recently been used for brucellosis.

Chapter 1

Introduction
1.6.2.3.6. *Adverse effect*

Rifampicin is usually well tolerated and rarely causes serious toxicity. The commonest side effects involve skin and gastrointestinal system.

- **Body fluids:** Tear, sweat and urine may become orange colored.
- **Skin:** Usually mild and self-limiting flushing and itching with or without rashes.
- **Gastrointestinal:** Lose of appetite, vomiting, abdominal pain and diarrhoea.
- **Liver:** Hepatitis, particularly when rifampicin is given with isoniazid.
- **Blood:** Thrombocytopaenia.
- **Musculoskeletal:** Muscle weakness and myopathy are uncommon.

1.6.2.3.7. *Precautions*

- Non-hormonal contraceptive methods may be necessary in women of child bearing age when taking rifampicin, due to its effect on oral contraceptives.
- If used in pregnancy, it may be harmful without causing malformations. It may appear in breast milk.
- Baseline blood tests should be done in adults, including a blood count, renal and liver function tests. If there are significant abnormalities, these should be repeated during treatment. Caution should be taken when there is pre-existing liver disease or liver function abnormalities.

1.6.2.3.8. *Carcinogenicity*

One report showed that nasopharyngeal lymphoma may develop after therapy of two years for Pott's disease.

1.6.2.3.9. *Teratogenicity*

Teratogenicity effects noted in rodents treated with high dose 100 to 150 mg/kg bodyweight daily in rodents have been reported to cause cleft palate and spina bifida. Malformation and death have been reported in infants born to mothers exposed to rifampicin, although it was the same frequency as in the general population.

1.6.2.3.10. *Mutagenicity*

There is no report available about development of mutagenicity by using rifampicin.
1.6.2.3.11. Toxicity

**Human data**

Patients have survived overdose of 12 g. The signs and symptoms are vomiting, lethargy, obtundation, seizures, pruritus, facial oedema, abdominal pain, pink to red discoloration of the skin, red color urine.

1.6.2.3.12. *Doses and administration*

Rifampicin is administered orally. When orally not feasible the drug is given I.V. infusion. It should not be given I.M. or subcutaneously.

**Adults**

**Oral:** 10 mg/kg bodyweight single daily dose (maximum 600 mg) in combination with other antimycobacterial agents.

**Parenteral:** Rifampicin has been administered intravenously at 20 mg/kg bodyweight (maximum daily dose 600 mg).

**Children:** 10 mg/kg bodyweight single daily dose in combination with other antimycobacterial agents.