“Colon-specific release of metronidazole from tablets compression-coated with different ratios of locust bean gum and carboxymethyl locust bean gum”

Thesis submitted for the Degree of Master of Pharmacy

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CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled "Colon-specific release of metronidazole from tablets compression-coated with different ratios of locust bean gum and carboxymethyl locust bean gum" has been carried out by Miss. Satarupa Bhattacharjee, M.Pharm, Jadavpur University, under my supervision in the division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, for partial fulfilment of the requirement for completion of the degree of Master of Pharmacy.

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DECLARATION OF ORIGINALITY

AND COMPLIANCE OF ACADEMIC ETHICS

I, hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of my Master of Pharmacy studies. All informations in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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Place: Jadavpur, Kolkata

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Dedicated to

my mother

Mrs. Shampa

Bhattacharjee

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PART I
INTRODUCTION

1.1. Introduction :

The major goal of any drug delivery system is to supply a therapeutic amount of drug to a target site in the body, so that the desired drug concentration can be swiftly achieved and then maintained for a desired period of time. Targeted drug delivery implies selective and effective localization of drug at the target site at therapeutic concentration with limited access to non target sites (1). A targeted drug delivery system is preferred for drugs having instability, low solubility, short biological half-life, large volume of distribution, poor absorption, low specificity and narrow therapeutic index (2). Targeted drug delivery may provide maximum therapeutic activity by preventing drug degradation or inactivation during transit to the target site. Besides, it can also minimize adverse effects caused due to inappropriate disposition and can also minimize toxicity of potent drugs by reducing their dose. An ideal targeted drug delivery system should be nontoxic, biocompatible, biodegradable and physicochemically stable both in-vitro and in-vivo (3). The preparation of such delivery system must be reasonably simple, reproducible and cost effective. The targeted drug delivery is dependent on the identification and exploitation of an attribute that is specific to the targeted organ.

Now a days drug delivery to colon is a thrust area of pharmaceutical research and is quite challenging in terms of overcoming the barriers of different pH regions in the gastrointestinal tract (G.I.T.) (4). The colon is the distal part of body’s digestive system which reabsorbs water, vitamins and minerals from food, then processes and stores wastes acting as faecal content storage reservoir until those wastes pass out of the body (5). The colon secrets $K^+$and HCO_3^-. It also hosts various friendly micro-organisms. The colonic region may be affected by various diseases. Usual drug delivery produces several serious systemic toxic effects in non targeted regions (6). Targeted drug delivery not only reduces the systemic side effects, but also provides an effective and safe therapy for colon related diseases with reduced dose (5).

Oral dosage forms of drug are the most reliable forms to the patients and widely accepted route of administration for delivery of active drug molecule. For successful therapy, the disease condition is not the only factor to consider but due attention should also be paid to the designing of suitable formulations.

1.2. Advantages of colon-specific drug delivery over conventional drug delivery systems :

There are various advantages of colon-specific drug delivery systems (CSDDS) over conventional ones as described below (7) :

- Colon targeted drug delivery, in treatment of local colonic diseases (e.g. constipation, spastic colon, Irritable Bowel Syndrome, Crohn's diseases, ulcerative colitis and colon cancer) is advantageous as it provides maximum therapeutic activity at the affected area by direct treatment, preventing degradation or inactivation of drug during transit to the target site.
- Colonic drug delivery needs administration of reduced dose of drugs and hence provides less local as well as less systemic side effects and also minimizes toxicity of potent drugs, as there is less inappropriate disposition.
- Colonic drug delivery helps in threshold entry of drugs, proteins and peptides into colon. These are enzymatically degraded or poorly absorbed in upper G.I.T. due to degradation in the acidic environment of stomach that can be prevented by developing a colon targeted delivery system. Colon is preferred to deliver proteins and peptides (e.g. oral vaccines, insulin, calcitonin, vasopressin, growth hormones etc.) in comparison to small intestine due to negligible activity of brush border membrane peptidase, much less activity of pancreatic enzymes.
- Colon is a potential site to achieve relevant bioavailability of poorly absorbed drugs, therapeutic proteins and peptides in particular. Therapeutic proteins and peptides show poor absorption from the upper G.I.T. because of their polar nature and/or their susceptibility to chemical or enzymatic degradation, such as high hepatic metabolism.
- Colon is an attractive site where poorly absorbed drug molecules may have an improved bioavailability.
- Colonic drug delivery is effective to treat serious diseases of the colon e.g. colorectal cancer.
- Colon-specific drug delivery systems reduce gastric irritation caused by many drugs e.g. NSAIDs and also clarify their mechanism of actions such as sulfasalazine which get metabolized in the colon to an active moiety 5-amino salicylic acid and interfere with the proliferation of colon polyps (first stage of colon cancer) probably in the local mode.

- Colon targeted drug delivery has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs.
- Colon targeting reduces fluctuation of dose levels.
- Colon-specific drug delivery improves treatment efficiency.
- Delay in absorption from such delivery system is desired from a therapeutic point of view in treatment of diseases that have peak symptoms in the early morning such as nocturnal asthma, angina and arthritis.
- Chronic colitis (ulcerative colitis, Crohn's disease) is currently treated with glucocorticoids and other anti-inflammatory agents. Administration of glucocortocoids namely dexamethasone and methyl prednisolone by oral and i.v routes produce systemic side effects including adenosuppresion, immunosuppresion, Cushinoid syndrome, bone resorption.
- Colon-specfic drug delivery improves patient compliance.
- Such targeted drug delivery systems are economical.

1.3. Anatomy and physiology of colon :

The anatomy and physiology of colon (Fig-1.1) is illustrated below (8) :

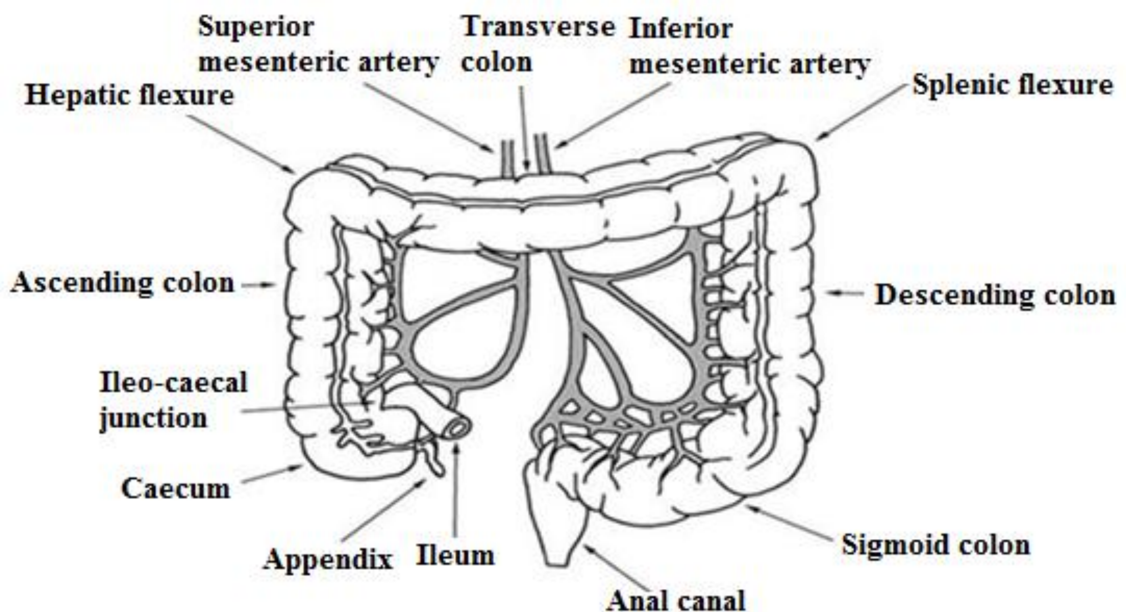

Fig-1.1. : Anatomy of colon

1.3.1. Large Intestine :

This is about 1.5 m long, beginning at the caecum in the right iliac fossa and terminating at the rectum and anal canal, deep in the pelvis. Its lumen is larger than that of the small intestine. The large intestine includes the following parts :

1.3.1.1. Caecum :

This is the first part of the colon. It is a dilated region which has a blind end inferiorly and is continuous with the ascending colon. It is usually about 13 cm long and has structure similar to the walls of the colon but contains more lymphoid tissue.

1.3.1.2. Colon :

This is the largest portion of the large intestine. The entire colon is about 5 ft (150 cm) long. The colon can be divided in four major segments :

i. Ascending Colon :

It travels upward to the diaphragm on the right side of the abdomen. It is 15 cm long.

ii. Transverse Colon :

It crosses the abdomen under the diaphragm. It is about 50 cm long.

iii. Descending Colon :

It travels downward through the abdomen on the left side. It is about 40 cm long.

iv. Sigmoid Colon :

It is S shaped region found in the lower abdomen.

1.4. Structure of colon :

The wall of colon (Fig-1.2) is consisting of four layers (9) :

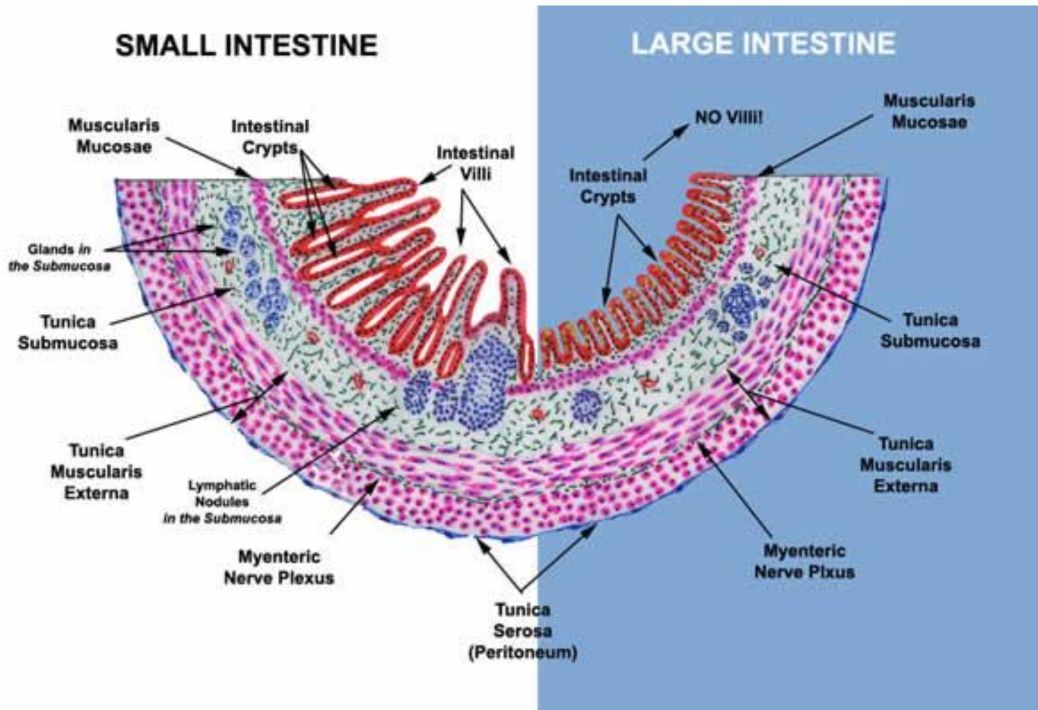

Fig-1.2. : Histological structure of colon

i. Serosa :

It is the exterior part of the large intestine which is the longitudinal muscle fibers spread out as can be found in the basic structure of G.I.T.

ii. Muscularisexterna :

It is the major muscular coat with some exceptions and consists of two layers of smooth (involuntary) muscle. The muscle fibers of the outer layer are arranged longitudinally and those of the inner layer encircle the wall of the tube. Between these two muscle layers there are blood vessels, lymph vessels and a plexus (network) of sympathetic and parasympathetic nerves, called the myenteric or Auerbach's plexus. These nerves supply the adjacent smooth muscle and blood vessels.

iii. Submucosa :

It is the connective tissue layer consisting of loose connective tissues with some elastic fibres. Within this layer there are plexuses of blood vessels, nerves, lymph vessels and varying amounts of lymphoid tissues. The blood vessels consist of arterioles, venules and

capillaries. The nerve plexus is the submucosal or Meissner's plexus, consisting of sympathetic and parasympathetic nerves which supply the mucosal lining.

iv. <u>Mucosa</u> :

It is the lining of the lumen of the colon, consisting of :

iv.i. <u>Epithelium</u> :

It is formed by columnar epithelium, the innermost layer that has three main functions : protection, secretion and absorption.

iv.ii. <u>Lamina propria</u> :

It is consisting of loose connective tissues, which support blood vessels that nourish the inner epithelial layer and also contains varying amounts of lymphoid tissues that have a protective role.

iv.iii. <u>Muscularis mucosae</u> :

It is a thin outer layer of smooth muscle that provides involutions of the mucosal layer e.g. gastric glands, villi.

Superior mesenteric artery supply blood to the proximal colon and the inferior mesenteric artery supply blood to distal colon.

1.5. pH of the G.I.T. :

The pH of the G.I.T. is subjected to both inter and intra subject variations. Diet, diseased state and food intake influence the pH of the gastrointestinal fluid. The changes in the pH along the G.I.T. have been used as a means of colon targeted drug delivery. Radio telemetry shows the highest pH (7.5±0.5) in the terminal ileum. On entry into the colon, the pH drops to 6.4±0.6. The pH in the mid colon is 6.6±0.8 and in the left colon 7.0±0.7 (10). There is a fall in pH on entry into the colon due to the presence of short chain fatty acids arising from bacterial fermentation of polysaccharides. For example lactose is fermented by the colonic bacteria to produce large amounts of lactic acid resulting in pH drop to about 5.0 (11). The pH the colon and the other part of the intestine are given below (Table-1.1).

Table-1.1. : Environment of various segments of G.I.T. :

G.I.T. segment	Length (m)	Surface area (m^2)	pH	Micro-organisms	Transit time (hr)	Internal diameter (cm)
1. Stomach	0.2	0.1	1.5	$\leq 10^2$	Variable	-
2. Small intestine						3.4
i. Duodenum	0.3	0.1	6.9	≤ 10	2	
ii. Jejunum	3.0	6.0	6.9	$\leq 10^5$	1.5	
iii. Ileum	4.0	6.0	7.6	$\leq 10^7$	1.5	
3. Large intestine	1.5	0.3	8.0	$\geq 10^{11}$	≤48	6

1.6. Colonic microflora and enzymes :

A large number of anaerobic and aerobic bacteria are present in the entire length of the human G.I.T. and produce various endogenous and exogenous substrates catalyzing enzymes. Over 400 distinct bacterial species have been found, 20-30% of which are of the genus bacteroids. The concentration of bacteria in the human colon is around 10^{11} to 10^{12} CFU/ml whereas in upper G.I.T. the concentration is 10^4 CFU/ml. The most important anaerobic bacteria are bacteroides, bifidobacterium, eubacterium, peptococcus, streptococcus, ruminococcus and clostridium (12). Summary of the most important metabolic reactions carried out by intestinal bacteria are given below (Table-1.2).

Table-1.2. : Metabolic reactions in colon :

Enzymes	Micro-organisms	Metabolic reactions catalysed
Nitro-reductase	E. coli. , bacteroids	Reduction of aromatic and heterocyclic nitrogeneous compounds
Azo-reductase	Clostridia, lactobacili, E. coli	Reductive cleavage of azo compounds

N-oxide reductase Sulfoxide reductase	E. coli	Reduction of N-oxide and sulfoxide
Esterase and Amidase	E. coli., P. vulgaris, B. subtilis	Cleavage of esters or carboxylic acid
Glucosidase	Clostridia, eubacteria	Cleavage of β-glucosidase of alcohol and phenol
Glucuronidase	E. coli, A. aerogenes	Cleavage of β-glucuronidase of alcohol and phenol
Hydrogenase	Clostridia, lactobacilli	Reduction of carbonyl groups and aliphatic double bonds
Sulfatase	Eubacteria, clostridia, streptococci	Cleavage of O-sulfates and sulfamates

1.7. Diseases in the colon :

Colon is prone to various diseases (Table-1.3) as described below (13-15). Drugs, selected for colon targeting are either able to treat colonic diseases or thay are from some different pharmacological classes and are unsuitable for delivery by other approaches (Table-1.4).

1.7.1. Inflammatory Bowel Disease (IBD) :

It refers to two chronic diseases that cause inflammation of the intestines : ulcerative colitis and Crohn's disease (Fig-1.3).

1.7.1.1. Ulcerative Colitis :

It is an inflammatory disease of the large intestine or colon. The inner lining (mucosa) of the intestine becomes inflammed (red and swollen) and develops ulcers (open painful wounds). It is often more severe in the rectal area, that can cause frequent diarrhoea. Mucous and blood often appear in the stool if the colon is damaged.

Drugs useful to treat ulcerative colitis are : balsalazine sodium, olsalazine sodium, mesalazine, budesonide and azathioprine.

1.7.1.2. Crohn's Disease :

It differs from ulcerative colitis by means of the affected areas of bowel. It affects terminal ileum and parts of large intestine but can attack any part of G.I.T. It causes inflammation that extends much deeper into the layers of intestinal wall whereas ulcerative colitis affects only the lining of the bowel. Crohn's disease causes ulceration and inflammation that affects the body's ability to digest food, to absorb nutrients and to eliminate waste in a healthy way.

Drugs useful to treat Crohn's disease are : prednisolone, azathioprine, budesonide, metronidazole, sulfasalazine, mesalazine and infliximab.

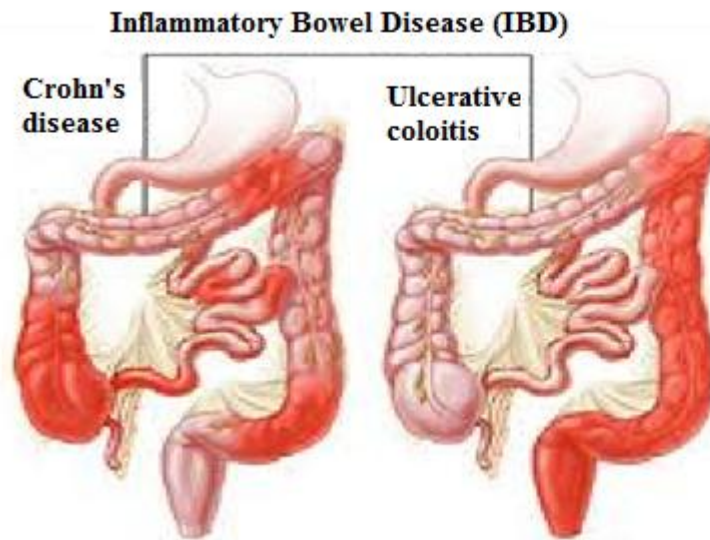

Fig-1.3. : Inflammatory Bowel Disease

1.7.2. Irritable Bowel Syndrome :

It is a chronic (long lasting) disorder of large intestine or colon. It is not a disease rather a functional disorder of bowel which can cause much distress but does not damage the bowel (Fig-1.4).

The most common symptoms are :

- Cramp and pain in abdomen, may be severe
- Constipation
- Diarrhoea
- Flatulance
- Bloating

- Feeling of fullness in rectum

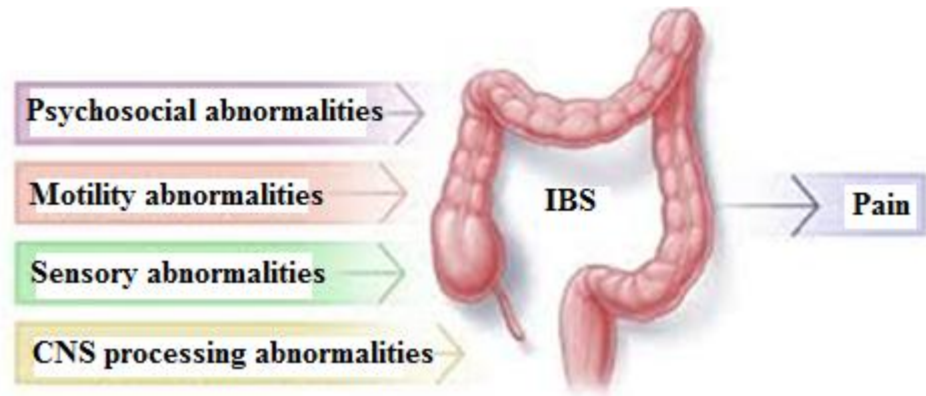

Fig-1.4. : Irritable Bowel Syndrome

1.7.3. Diverticular Disease :

It is related to large intestine especially colon. It is made up of two conditions : Diverticulosis and Diverticulitis (Fig-1.5).

1.7.3.1. Diverticulosis :

Here pouches (Diverticula) are formed in the colon that bulge out like weak spots in a tire.

The most common symptoms are :

- Cramp and pain
- Discomfort in lower abdomen
- Bloating
- Constipation

1.7.3.2. Diverticulitis :

Here those pouches become inflammed.

The most common symptoms are :

- Abdominal cramp and pain
- Nausea
- Vomiting
- Fever
- Chill

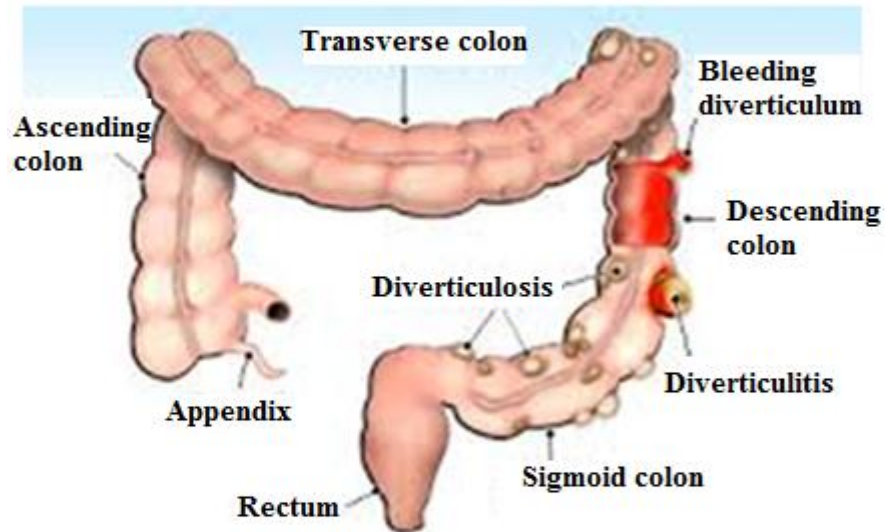

Fig-1.5. : <u>Diverticular disease</u>

1.7.4. <u>Colorectal Polyps</u> :

It is a polyp (fleshy growth) occuring on the lining of the colon or rectum (Fig-1.6). Untreated ones can develop colorectal cancer. These are often classified as :

- Benign (e.g. Hyperplastic polyp)
- Pre malignant (e.g. Tubular adenoma)
- Malignant (e.g. Colorectal adenocarcinoma)

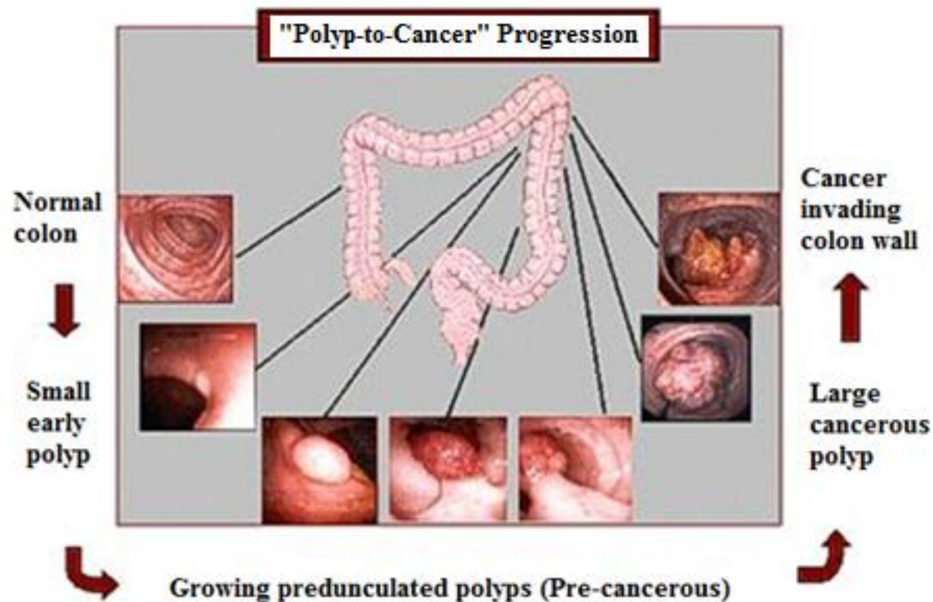

Fig-1.6. : <u>Colorectal polyps</u>

1.7.5. Colon Cancer :

It, also known as rectal cancer, bowel cancer or colorectal adenocarcinoma, develops due to uncontrolled cell growth in the colon or rectum or appendix (Fig-1.7).

The most common symptoms are :

- Rectal bleeding
- Anaemia
- Weight loss
- Changes in bowel habits

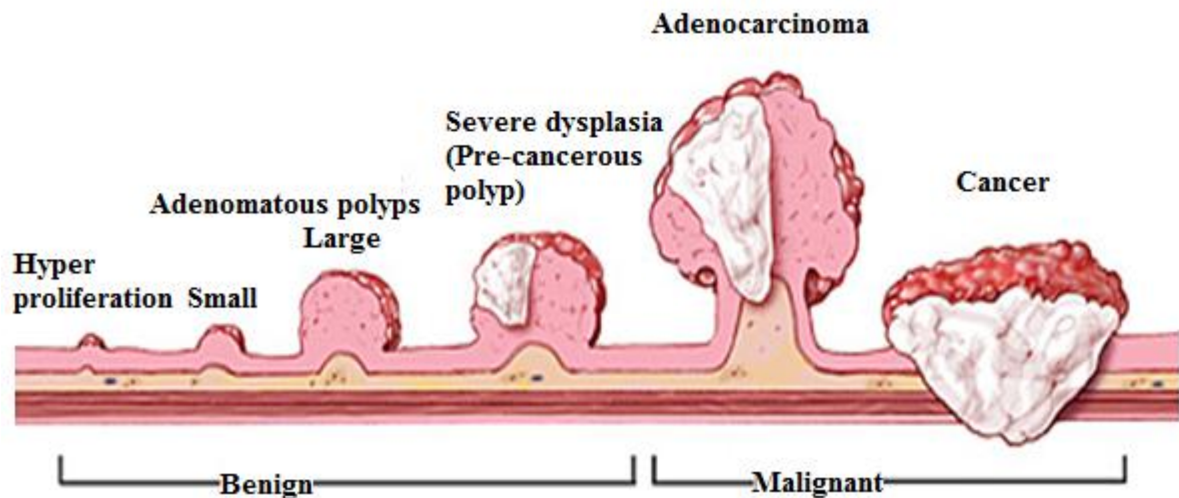

Fig-1.7. : Colon cancer

1.7.6. Infections :

The colon is a common site of infection for a heterogeneous group of bacterial pathogens like Salmonella, E. coli, Staphylococci which cause infections, amoebic dysentery and bacillary dysentery.

i. Amoebiasis :

An infection or disease, caused by amoeba, especially of the species *Entamoeba histolytica* characterized by dysentery. Amoebiasis most commonly occur in young to middle aged persons.

ii. Diarrhoea :

Stools that are frequent, loose or watery are commonly called diarrhoea. Most diarrhoea is due to self limited, mild infections of the colon or small intestine.

iii. Salmonellosis :

The bacteria salmonella can contaminate food and infect the intestine. Salmonella causes diarrhoea and stomach cramps, which usually resolve without treatment.

iv. Shigellosis :

The bacteria shigella can contaminate food and invade the colon. Symptoms include fever, stomach cramps and diarrhoea, which may be bloody.

v. Traveler's diarrhoea :

Many different bacteria commonly contaminate water or food in developing countries. Symptoms are loose stools, sometimes with nausea and fever.

Table-1.3. : Colon targeting sites, diseases and drugs :

Target sites	Disease conditions	Drug and active agents
Topical action	Irritable Bowel Syndrome, Crohn's disease and chronic pancreatitis	Hydrocortisone, budesonide, prednisolone, sulfasalazine, olsalazine, mesalazine, balsalazide
Local action	Pancreatectomy, cystic fibrosis and colorectal cancer	Digestive enzyme supplements, 5-fluorouracil
Systemic action	• To prevent gastric irritation • To prevent first pass metabolism of orally ingested drugs • Oral delivery of peptides • Oral delivery of vaccines	NSAIDs Steroids Insulin Typhoid

Table-1.4. : Criteria for selection of drugs for CSDDS :

Criteria	Pharmacological class	Non-peptide drugs	Peptide drugs
Drugs used for	Anti-inflammatory	Oxyprenolol, metoprolol,	Amylin, antisense

local effects in colon against G.I.T. diseases	drugs	nifedipine	oligonucleotide
Drugs probably absorbed from upper G.I.T.	Anti-hypertensive and anti-anginal drugs	Ibuprofen, isosorbides, theophylline	Cyclosporin, desmopressin
Drugs for colon cancer	Anti-neoplastic drugs	Pseudoephedrine	Epoetin, glucagon
Drugs that degrade in stomach and small intestine	Peptides and proteins	Bromopheneramine, 5-fluorouracil, doxorubicin	Gonadoreline, insulin, interferons
Drugs that undergo extensive first pass metabolism	Nitroglycerin and corticosteroids	Bleomycin, nicotine	Protirelin, sermorelin, saloatonin
Drugs for targeting	Anti-arthritic and anti-asthamatic drugs	Prednisolone, hydrocortisone, 5-amino salicylic acid	Somatropin, urotoilitin

1.8. Transit of material through the colon :

The presence of food generally increases gastric residence. In some cases with regular feeding, dosage forms have been shown to reside in the stomach for periods in excess of 12 hours (16-18). Small intestinal transit is surprisingly constant at 3 to 4 hours and appears to be independent of the type of dosage form and whether the subject is in the fasted or fed state (17). Compared to other regions of the G.I.T., movement of materials through the colon is slow. The total time for transit tends to be highly variable and influenced by a number of factors such as diet, in particular dietary fibre content, mobility, stress, diseases and drugs (19). Colonic transit times ranged from 50 to 70 hours. Stool weights increased significantly

with the presence of active disease presumably due to exudates from inflamed epithelium, increased mucus secretion and reduction in reabsorption of fluid and electrolytes.

1.9. Drug absorption in the colon :

Drugs are generally absorbed from the colon by paracellular or transcellular pathway. Lipophilic and hydrophilic drugs pass through transcellular and paracellular routes respectively. Sometimes poor absorption of many drugs through the paracellular route is due to the tight junction. Theoretically, drug absorption can occur along the entire G.I.T., while actually, most drugs are absorbed in the duodenum and proximal jejunum.

1.10. Factors affecting absorption of drug in colon :

Many other parameters influence the bioavailability of drug through the colon such as (20-22) :

1.10.1. Physicochemical parameters :

1. Lipophilicity :

The lipophilicity means lipid solubility of drug which helps to permeate through the lipoidal biological membrane and is denoted as logP. In another term it is called as partition co-efficient of drug. Lipophilicity can indicate whether the drug is absorbable from the G.I.T. or not.

- logP less than zero indicates that the drug is more hydrophilic and will be absorbed from the G.I.T. by paracellular route, if the drug molecules are smaller in size. They should be preferably administered by parenteral route.
- logP greater than zero but less than three indicates good absorption from G.I.T. upon oral administration as their lipophilic and hydrophilic groups are balanced .
- logP more than three indicates well absorption from G.I.T. as they are more lipophilic in nature but will be localized in the fatty tissues and are susceptible to metabolism and biliary clearance.

2. Molecular weight :

Molecular weight is another important factor which determines absorption of drug molecules. Study showed that :

- Molecular weight less than or equal to 500 Dalton indicates absorption through transcellular passive diffusion.
- Molecular weight near about 200 Dalton indicates absorption through paracellular diffusion.

3. H-bond :

To be well absorbable from the G.I.T. the drug molecule should contain :

- Not more than ten H-bond acceptors.
- Not more than five H-bond donors.

4. pKa value :

Since most of the drugs are weak electrolytes (weak acids or weak bases), their degree of ionization depends upon the pH of the biological fluid. In case of weakly acidic and weakly basic drugs, the pKa value is important for prediction of solubility. Total solubility will be increased when $pH \gg pKa$ of weakly acidic drugs and $pH \ll pKa$ of weakly basic drugs.

5. Drug stability :

A drug for oral use may destabilize either during its shelf-life or during transit in the G.I.T. Two major stability problems resulting in poor bioavailability of an orally administered drug are degradation of the drug into inactive form and interaction of the drug molecule with one or more different components either of the dosage form or those present in the G.I.T. like bile, mucous that form a complex which is poorly soluble or is unabsorbable.

1.10.2. Pharmaceutical parameters :

1. Drug candidate :

Drugs which show poor absorption in the stomach and small intestine are most suitable for colon delivery. Drugs such as theophylline, nifedipine, ibuprofen, diclofenac, metoprolol, isosorbide dinitrate, oxyprenolol and low molecular weight peptides and peptide like drugs have been shown to be effectively absorbed from the colon.

2. Drug carrier :

The selection of carrier for a particular drug candidate depends on the physicochemical nature of the drug as well as the disease for which the system is to be used. The factors such as chemical nature, stability and partition co-efficient of drug and the type of absorption enhancers influence the carrier selection. The carrier which contains additives like polymers may influence the release property.

1.10.3. Patient related parameters :

1. Gastric emptying :

Apart from dissolution of a drug and its permeation through the biomembrane, the passage from the stomach to the intestine, called as gastric emptying can also be a rate-limiting step in drug absorption because the major site for drug absorption is small intestine.

A large number of factors influence gastric emptying :

- Volume of meal
- Composition of meal
- Emotional state
- Body posture

2. Age :

There is a significant variation in the gastric pH and intestinal surface area and blood flow in the G.I.T. between the infants and adults. In infants the gastric pH is high and intestinal surface area and blood flow to the G.I.T. is low resulting in altered absorption pattern in comparison to adults. In elderly persons, altered gastric emptying and low G.I.T. blood flow causes impaired drug absorption.

3. Gastrointestinal pH :

A tremendous difference (107 fold) in the hydrogen ion concentration is observed between the gastric and colon fluids. The gastrointestinal pH generally increases gradually as one move down from the stomach to the colon and rectum. This alteration in the pH leads to :

- Earlier disintegration
- Earlier dissolution
- Destabilization of the dosage form

Also for treatment of these local diseases, drug targeting not only reduces dose of the drug to be administered, but also reduces the incidence of possible adverse effects associated with chemotherapeutic agents. The most critical challenge in such drug delivery approach is to preserve the formulation during its passage through the stomach and small intestine. The concentration of drug reaching the colon depends on formulation factors, the extent of retrograde spreading and the retention time. Foam and suppositories have been shown to be retained mainly in the rectum and sigmoid colon while enema solutions have a great spreading capacity (23) implying efficacy of the system.

1.11. Existing colon-specific drug delivery systems :

Several approaches are used for site-specific drug delivery. Among the primary approaches for CSDDS, the existing drug delivery systems are (24-27) :

1. Primary approaches for CSDDS :

1.1. pH dependent drug delivery :

i. pH-sensitive polymer coating :

In the stomach, pH ranges between 1 to 2 during fasting but increases after eating. The pH is about 6.5 in the proximal small intestine, and about 7.5 in the distal small intestine. From the ileum to the colon, pH declines significantly. It is about 6.4 in the cecum. However, pH values as low as 5.7 have been measured in the ascending colon in healthy volunteers. The pH in the transverse colon is 6.6 and 7.0 in the descending colon. Use of pH dependent polymers is based on these differences in pH levels. The polymers described as pH dependent in colon-specific drug delivery systems are insoluble at low pH levels but become increasingly soluble as pH rises. The intact drug molecule can be delivered to the colon without absorbing in the upper G.I.T. by the help of a coating with suitable polymers around the drug molecules present in the core in such a way that the coating will degrade only in the colon. The core includes tablets, capsules, pellets, granules, microparticles or nanoparticles. The coating of pH-sensitive polymers to the tablets, capsules or pellets provide delayed release and protect the active drug from gastric fluid. A careful selection of enteric coating material and thickness is needed to ensure that disintegration does not occur until the dosage

from moves through the ileocecal junction from the terminal ileum into the caecum. A delayed release tablet formulation containing mesalazine coated with eudragit S-100 is dissolved at a pH ≥7, releasing mesalazine in the terminal ileum and colon for topical inflammatory action.

Disadvantages of pH sensitive polymer coating are :

- Although a pH dependent polymer can protect a formulation in the stomach and proximal small intestine, it may start to dissolve in the lower small intestine.
- The site specificity of formulations can be poor. The decline in pH from the end of the small intestine to the colon can also result in problems. Lengthy lag times at the ileocecal junction and rapid transit through the ascending colon can also result in poor site-specificity of enteric-coated single-unit formulations.

ii. <u>pH-sensitive embedded matrices</u> :

As the drug has to reach in the colon to treat the local colonic diseases, it should not be susceptible to degradation in the acidic environment of the stomach. Before reaching to the colon, the dosage forms must pass through the stomach (pH≈1.5-3.5), the duodenum (pH≈6), the large intestine (pH≈5.5-6.8), the caecum (pH≈6.8-7.3) preventing drug release upto this and should release the entire amount of drug in the colon (pH≈6.4).

1.2. <u>Delayed (Time controlled release system) release drug delivery to colon</u> :

Time controlled release system (TCRS) such as sustained, delayed or pulsatile release dosage forms are also very promising drug release systems although not ideal to deliver drugs into the colon, as there is potentially large variations in gastric emptying time of dosage forms in humans. In these approaches (Fig-1.8), colon arrival time of dosage forms cannot be accurately predicted, resulting in poor colonical availability. This approach is based on the principle of delaying the drug release until it enters into the colon. In this approach, drug is released from the dosage form after a pre-determined lag time according to the transit time from mouth to colon. The lag time depends upon the gastric motility and size of the dosage form. A lag time of 5 hours is usually considered sufficient since small intestine transit is about 3 to 4 hours, which is relatively constant and hardly affected by the nature of formulation administered. Time controlled systems are useful for synchronous delivery of a drug at a pre-determined time such that patient receives the drug when needed and at a pre-

selected site. These systems are therefore particularly useful in the therapy of diseases, which depend on circadian rhythms.

Disadvantages of time controlled release systems are :

- Gastric emptying time varies markedly depending on type and amount of food intake.
- Gastrointestinal movement, especially peristalsis or contraction in the stomach may result in change in gastrointestinal transit of the drug.
- Accelerated transit through different regions of the colon has been observed in patients with IBD, diarrhoea and carcinoid syndrome.
- Colon arrival time of dosage forms can not be accurately predicted which result in poor colonical activity.

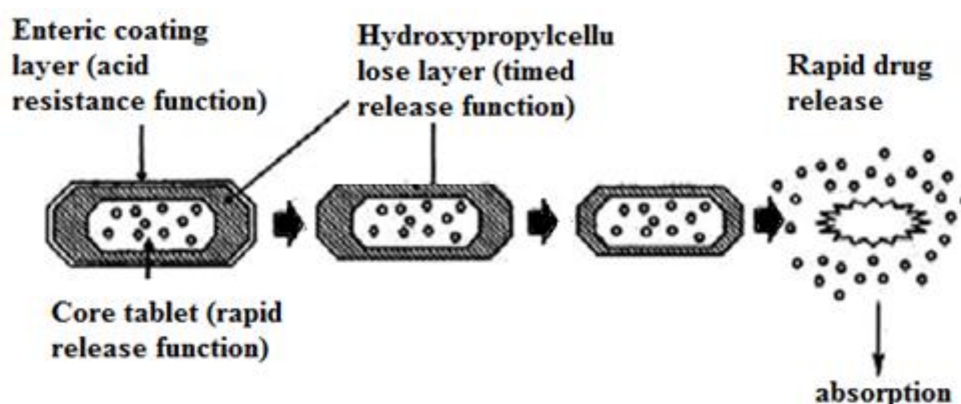

Fig-1.8. : Design of enteric coated time release press-coated tablet

1.3. Microbially triggered drug delivery to colon :

The resident bacteria present in the G.I.T. affect drug release in the colon. These bacteria predominantly reside at the distal region of G.I.T. The bacterial count in the colon is around 10^{11} to 10^{12} CFU/ml as compared to 10^{4} CFU/ml in upper G.I.T. Moreover, 400 different species are present in the G.I.T., containing mainly of anaerobic bacteria e.g bacteroides, bifidobacteria, eubacteria, clostridia, enterococci, enterobacteria, pneumococcus and ruminococcus etc. This vast microflora fulfils its energy needs by fermenting various types of substrates that have been left undigested in the small intestine, e.g. di- and tri-saccharides, polysaccharides etc. For this fermentation, the microflora produces a vast number of enzymes like glucoronidase, xylosidase, arabinosidase, galactosidase, nucleoreductase,

azoreductases, urea dehydroxylase and deaminase. These are released by the colonic bacteria that are capable of metabolizing endogenous and exogenous substrates, such as carbohydrates and proteins. Because of the presence of enzymes in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be more site-specific approach as compared to other approaches. These polymers shield the drug from the environment of stomach and small intestine and are able to deliver the drug to the colon specifically. Hence these polymers can also be utilized as a drug carrier.

Advantages of this type of drug delivery include :

- Because of the presence of the enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be a more site-specific approach as compared to other approaches.
- These polymers shield the drug from the environment of stomach and small intestine, and are able to deliver the drug to the colon specifically. On reaching to the colon, they undergo assimilation by micro-organisms, or degradation by enzymes. Break down of the polymer backbone leads to reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer.

However, the principal disadvantage of this system is that :

- The content of colonic microfloral enzymes that are essential to cleave polymeric backbone, vary considerably with age, diseased state, food habit and antibiotic therapy.

1.3.1. <u>Prodrug approach for drug delivery to colon</u> :

Prodrug is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation in-vivo to release the active drug. For colonic delivery, the prodrug is designed to undergo minimal hydrolysis in the upper G.I.T., and undergoes enzymatic hydrolysis in the colon thereby releasing the active drug moiety from the drug carrier (Table-1.5). Metabolism of azo compounds by intestinal bacteria is one of the most extensively studied bacterial metabolic process. A number of other linkages susceptible to bacterial hydrolysis specially in the colon have been studied where the drug is attached to hydrophilic moieties like amino acids, glucoronic acids, glucose, galactose, cellulose etc. This

process of biotransformation is carried out by a variety of enzymes, mainly of bacterial origin, present in the colon. The enzymes that are mainly used for colon targeted drug delivery include azoreducatase, galactosidase, β-xylosidase, nitroreductase, glycosidase,deaminase etc.

Disadvantages are :

- It is not a very flexible approach as its formulation depends upon the availability of functional group on the drug moiety, required for chemical linkage.
- Prodrugs are new chemical entities and need a lot of evaluation before using as carriers.

Table-1.5. : Prodrugs evaluated for colon-specific drug delivery with their in-vitro/in-vivo performance :

Carrier	Drug investigated	Linkage hydrolysed	In-vitro/in-vivo model used	Performance of the prodrug/conjugates
Azo conjugates sulphapyridine (SP)	5-ASA	Azo linkage	Human	It is site-specific with a lot of side effects associated with SP
5-ASA	5-ASA	Azo linkage	Human	It delivers 2 molecules of 5-ASA
Glycine conjugates	Salicylic acid	Amide linkage	Rabbit	It was absorbed from upper G.I.T., though metabolized by microflora of large intestine
Tyrosine/methionine conjugates	Salicylic acid	Amide linkage	Rabbit	It was absorbed from upper G.I.T., though metabolized by microflora of large intestine

L-Alanin/D-Alanin conjugates	Salicylic acid	Amide linkage	In-vitro	Salicylic acid-L-Alanine was hydrolysed to salicylic acid by intestinal micro-organisms but Salicylic acid-D-Alanine showed negligible hydrolysis thereby showing enantio-specific hydrolysis
Glycine	5-ASA	Amide linkage	In-vitro	Prodrug was stable in upper G.I.T. and was hydrolysed by caecal content to release 5-ASA
Saccharide carriers	Dexamethasone/ prednisolone	Glycosidic linkage	Rat	Dexamethasone prodrug was site-specific and 60% of oral dose reached to the caecum. Only 15% of prednisolone prodrug reached to the caecum
Glucose/galactose/cellobioside	Dexamethasone, prednisolone, hydrocortisone, fludrocortisone	Glycosidic linkage	In-vitro	Less hydrolysis of the prodrug was seen in contents of stomach and proximal small intestine. Hydrolysis increased in contents of distal small intestine and was maximum in caecal content homogenates. Galactosides were hydrolysed faster than glucosides which were again hydrolysed faster than the corresponding cellobioside

Glucuronic acid conjugates	Naloxone/nalmefene	Glucuronide linkage	Rat	When given to morphine dependent rats, these reversed the G.I.T. side effects caused by morphine without causing CNS withdrawal symptoms because of its activation in large intestine followed by a resultant diarrhoea which excreted the prodrug
	Budesonide	Glucuronide linkage	Rat	It was found to be superior than budesonide itself for the treatment of colitis

1.3.1.1. <u>Azo bond conjugate</u> :

This approach is based on the conjugation of azo bond. The colonic bacteria extensively metabolize these azo compounds through the intracellular and extracellular reduction. The azobond is stable in the upper G.I.T. and is cleaved in the colon by the azo-reductases produced by the microflora (Table-1.6). An excellent example of azo bond conjugate is sulfasalazine (Fig-1.9). Sulfasalazine is mainly used for the treatment of IBD. It is 5-amino salicylic acid (5-ASA) conjugated with sulphapyridine that is a carrier for 5-ASA. About 85% of oral dose of sulfasalazine reaches to the colon unabsorbed, where it is reduced in the anaerobic environment into 5-ASA and sulphapyridine (28,29).

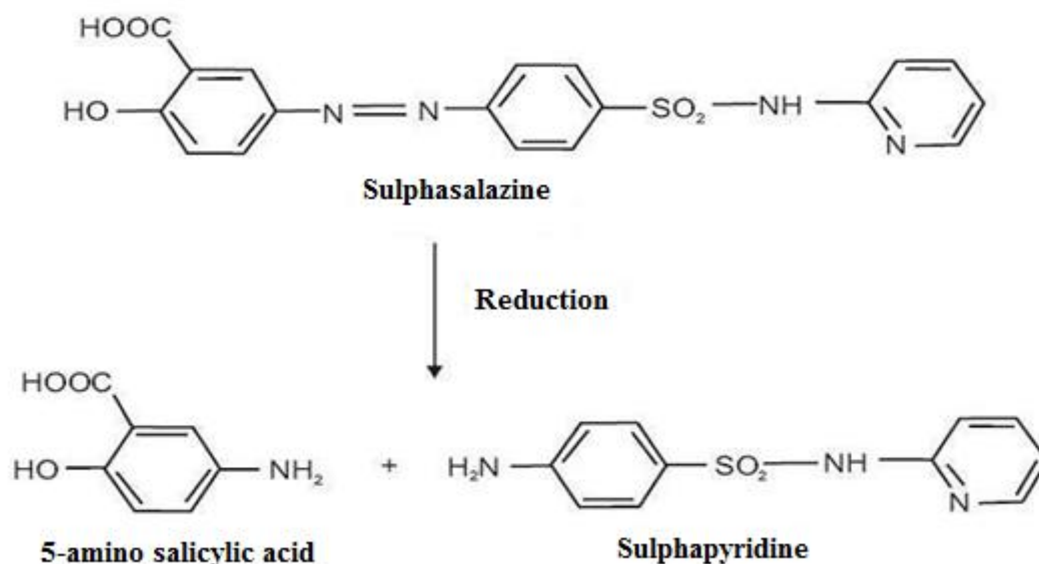

Fig-1.9. : Reduction reaction of sulphasazine in 5-ASA and sulphapyridine

Table-1.6. : Some azopolymer based drug delivery systems for CSDDS with summarized results :

Azo polymer	Dosage form prepared	Drug investigated	In-vitro/in-vivo model used	Summary of the results obtained
Copolymers of styrene with 2-hydroxyethyl methacrylate	Coating over capsules	Vasopressin, insulin	Rats	These capsules showed biological response characteristics of these peptide hormones in dogs though it varied quantitatively
Hydrogels prepared by copolymerization of 2-hydroxyethyl methacrylate with 4-methacryloxy azobenzene	Hydrogel	5-fluorouracil	Dogs	Drug release was faster and greater in human caecal media compared to simulated gastric and intestinal fluids

Segmented polyurethanes	Coating over pellets	Budesonide	In-vitro	These azopolymer coated pellets were useful for colon-specific delivery of budesonide to heal induced colitis
Aromatic azo bond containing urethane analogues	Degradable films	5-amino salicylic acid	In-vitro degradation of films in presence of lactobacilus	These films were degraded by azo-reductase. The permeability of 5-ASA from lactobacilus treated films was significantly higher than that of control

1.3.1.2. <u>Glycoside conjugation</u> :

This approach is been based upon the unique glycosidase activity of the colonic microflora. Certain drugs can be conjugated to different sugar moieties to form a glycoside. The drug moiety forms aglycone and sugar part forms glycone of the glycoside. These glycoside molecules are hydrophilic in nature and thus impermeable to the biological membrane upon ingestion. They are broken down upon the action of glycosidase, releasing the drug from the sugar. These conjugates can be expected to be good colon-specific drug carriers. The major glycosidase enzymes produced by the intestinal microflora are : β-D-galactosidase, α-L-arabinofuranosidase, β-D-xylopyranosidase, β–D-glucosidase.

These glycosidase enzymes are located at the brush border and hence are easy accessible to substrates.

1.3.1.3. <u>Glucoronide conjugation</u> :

Bacteria present in the lower G.I.T. secrete β-glucuronidase, which can deglucuronide a variety of drugs in the colon. The glucuronidation process results in the release of active

drug and enables its reabsorption. So the glucuronide prodrugs are expected to be superior for colon targeting (30). Opiates, when taken for the relief of pain, can cause severe constipation by inhibiting gastrointestinal motility and secretion. Narcotic antagonists, when given as an antidote for gastrointestinal side effects, immediately relieve constipation but precipitate acute withdrawal symptoms. A novel approach will be to target these antagonists to the lower bowel so that they are not absorbed systemically. With this purpose, naloxone and nalmefene glucuronide prodrugs were prepared to target these drugs to the colon.

1.3.1.4. Cyclodextrin conjugation :

This approach is based on the potential of cyclodextrin to form inclusion complex with the drug that improves solubility, stability and bioavailability of the drugs.

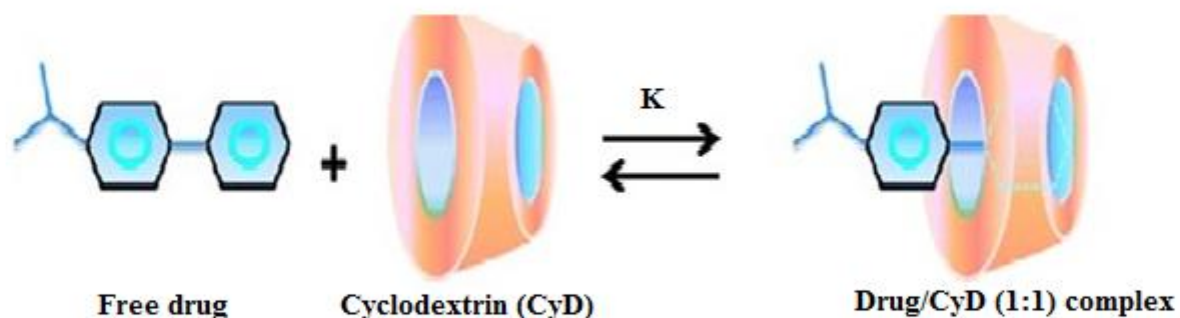

Fig-1.10. : Formation of cyclodextrin inclusion complex

Cyclodextrins are mainly cyclic oligosaccharides consisting of 6 to 8 glucose units linked through α-1,4 glucosidic bonds. The interior portion of these molecules is relatively lipophilic and the exterior part is relatively hydrophilic (Fig-1.10). By virtue of which they are less capable of being hydrolysed and are only slightly absorbed during passage through the stomach and small intestine. However, colonic bacteria are capable of degrading cyclodextrins, a carbon source, by stimulating cyclodextranase activity. They are fermented by the colonic microflora to form monosaccharides that are then absorbed. The α- and β-cyclodextrins are practically resistant to gastric acid and salivary and pancreatic amylases. Methotrexate prodrugs of α- and γ-cyclodextrins were synthesized in order to mask the ulcerogenic potential of free drug by using 12 fold dose of a normal dose of methotrexate and equivalent doses of the esters (24).

1.3.1.5. Dextran conjugation :

This approach is based upon dextran polysaccharides that mask the drug in the upper G.I.T. and in presence of dextranase, releases the drug in the colon. The dextranase activity is shown by anaerobic gram negative bacteria, especially the bacteroides, which are present in a concentration as high as 10^{11} CFU/ml in colon. Dextran prodrug approach can be used for colon-specific delivery of drugs containing a carboxylic acid function (−COOH).

1.3.1.6. Amino acid conjugation :

Hydrophilic nature of polar groups like -NH2 and -COOH, that are present in the proteins and their basic units (i.e. amino acids), reduce the membrane permeability of amino acids and proteins. Various prodrugs have been prepared by the conjugation of drug molecules with these polar amino acids. Non-essential amino acids such as tyrosine, glycine, methionine and glutamic acid were conjugated to salicylic acid (31).

1.3.2. Azo-polymeric prodrugs :

Newer approaches are aimed at the use of polymers as drug carriers for drug delivery to the colon. Both synthetic as well as naturally occurring polymers have been used for this purpose. Semi-synthetic polymers have been used to form polymeric prodrug with azo linkage between the polymer and drug moiety. These have been evaluated for CSDDS. Various azopolymers have also been evaluated as coating materials around drug cores. These have been found to be similarly susceptible to cleavage by the azo-reducatase in the large intestine. Coating of peptide capsules with polymers, cross-linked with azo-aromatic group have been found to protect the drug from digestion in the stomach and small intestine. In the colon, the azo bonds are reduced and the drug is released.

1.3.3. Polysaccharide based delivery systems :

Recently natural polymers have gained much popularity for the development of colon-specific drug delivery systems specially in the form of compression coated tablets. For targeted delivery of active moieties to the colonic part of the G.I.T., polymers are a choice for dosage form formulation. In the swollen state, the polymers become soft and rubbery and they resemble living tissues exhibiting excellent biocompatibility (32). The polymers can be formulated as solid, liquid and semi-solid dosage forms. Formulation development of dosage

forms, using various natural polymers that will not be degraded in acidic pH of the upper G.I.T., can be a suitable approach of preventing drug release during the transit time in upper G.I.T.

Polymers can be categorized into two groups such as natural and synthetic polymers (Table-1.7).

Table-1.7. : Classification of polymers :

Natural polymer		Synthetic polymer	
Plant origin	**Animal origin**	**Inorganic**	**Organic**
• Cellulose • Hemicellulose • Glucomannan • Agar • Starch • Pectin • Inulin • Rosin • Guar gum • Locust bean gum • Gum acacia • Gum tragacanth • Gum arabic • Karaya gum • Aloe vera gel	• Chitin • Alginates • Carageenans • Psyllium • Xanthan gum	• Polysiloxane • Polyphosphazene	• Low density polyethylene • High density polyethylene • Polypropylene • Poly vinyl chloride • Polystyrene • Nylon • Teflon • Thermoplastic polyurethanes

Locust bean gum (LBG), also known as Carob gum, is obtained from the endosperms of the seeds of *Ceratonia siliqua*, family Leguminosae (Fig-1.11). It is composed of galactomannans, which are a series of natural polysachharides from various sources sharing a similar chemical structure. It is a non gelling, nonionic, natural polysaccharide. The main chain consists of 1,4-linked β-D-mannopyranose backbone with branch points from their six positions linked to single α-D-galactose residue (1,6-linked α-D-galactopyranose) (33-35)

(Fig-1.12). Locust bean gum has an overall ratio of mannose to galactose of around 4:1. The galactose sugars are not evenly distributed along the chain but tend to be clustered together in blocks. The chains have an irregular structure with alternating "smooth" and substituted zones. LBG being a polymer is a macromolecule and consists of very large chain, functional groups and can be blended with other low and high molecular weight materials. Thus LBG can be tailored for any application for delivery of drugs to the target site.

Fig-1.11. : Ceratonia pods, seeds and dried powder

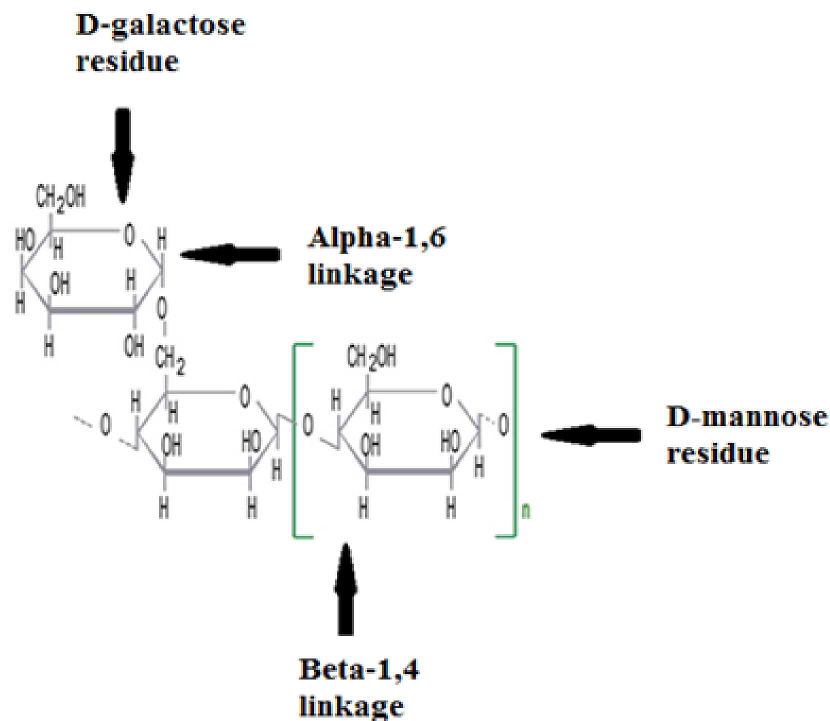

Fig-1.12. : Chemistry of locust bean gum

Advantags of polysachharides are :

- The polymers are hydrophilic, hydrosoluble in nature.
- Polymers are found in abundance. They are variable, inexpensive and available in various structures with variety of properties.
- The polymers can be easily modified chemically and biochemically. They are highly stable, gel forming and biodegradable, which suggest their use in targeted drug delivery systems.
- The pharmaceutical applications of polymers range from their use as binders in tablets to viscosity and flow controlling agents in suspensions and emulsions. Polymers can be used as film coating materials to disguise the unpleasant taste of a drug, to enhance drug stability, to modify drug release characteristics (e.g. delayed, sustained, pulsatile etc.) and to improve bioavailability. The rate of the drug release from a compression coated tablet depends on the initial drug concentration and relaxation of the polymer chains in the coat, which overall display a pulsatile release characteristic.
- Polymers from natural sources are non-toxic, stable, safe and without side effects.
- Natural polysaccharides can be broken down by the colonic microflora to simple saccharides.
- In many countries, polymers are produced for industrial applications (36).
- Polymers are cheaper and their production cost is less than synthetic materials.

Polysaccharides provide several benefits as carrier molecules or encapsulation materials. They generally have a predictable degradation pattern in the G.I.T., allowing consistent drug release. Polysaccharides also hydrate and swell during their transit through the G.I.T., creating a barrier against diffusion of the drug. Upon arrival to the colon, colonic bacteria and enzymes are able to degrade the polysaccharide coats to release the drug (37). The use of naturally occurring polysaccharides is attracting a lot of attention for drug targeting to the colon (Table1.8). These include naturally occurring polysaccharides obtained from plant (guar gum, inulin), animal (chitosan, chondrotin sulphate), algal (alginates) or microbial (dextran) origin. The polysaccharides can be broken down by the colonic microflora to simple saccharides. Therefore, they fall into the category of “Generally Regarded As Safe” (GRAS).

Table-1.8. : Polysaccharides investigated for colon targeting with their dosage forms and summarized results :

Polysaccharide investigated	**Drug moiety used**	**Dosage form prepared**	**In-vitro/in-vivo model used**	**Performance of the system**
Chitosan	5-(6) carboxy fluorescein (CF)	Enteric coated chitosan capsules	In-vitro	Little release of CF in upper G.I.T.and 100% drug release in 33% caecal content within 4 hours of dissolution
Chitosan phthalate, Chitosan succinate	Sodium diclofenac	Matrices	In-vitro	Reduced drug release in acidic conditions and improved dissolution in basic conditions
Calcium salt of pectin	Indomethacin	Matrices	In-vitro	In the presence of rat caecal content drug release was 60.8±15.7% as compared to 4.9±1.1% in control
Amidated pectin	Paracetamol	Matrix tablets	In-vitro	These matrices were not suitable for colonic drug delivery
Amidated pectin/calcium pectinate	Ropivacaine	Matrix tablets with ethylcellulose as drug matrix additive	In-vitro	These were more susceptible to pectinolytic enzymes as compared to calcium pectinate. Addition of ethylcellulose increased tablet strength and dissolution rate. Coating with eudragit L100 reduces drug release in upper G.I.T. without effect of enzymatic degradation

Chondroitin sulphate	Indomethacin	Matrix tablets	In-vitro	Drug release increases in presence of rat caecal content while it decreases with increase in cross-linking
Calcium salt of cross-linked chondroitin alginate	5-ASA	Double coated swellable beads	In-vitro	Enteric coating dissolves in basic media, beads swell to exceed the strength of aquacoat and drug releases subsequently
Guar gum and xanthan gum	Naproxen	Compression-coated matrix tablets	In-vitro	It was found that 0 to 7.8% drug was released in upper G.I.T., while 66.40 to 97.82% drug was released in simulated colonic fluid
Xanthan gum and hydroxypropylmethyl cellulose	Meloxicam	Compression-coated tablets	In-vitro and in-vivo	In-vitro study results showed that there was no drug release in stomach and small intestine while in-vivo study showed that the tablets reached to the colon
Locust bean gum, guar gum, xanthan gum and hydroxypropylmethyl cellulose	Aceclofenac	Compression-coated tablets	In-vitro	Restricted drug release in upper G.I.T. and increase in drug release in presence of 4% rat caecal content.

2. Newly developed approaches for CSDDS :

2.1. Pressure controlled drug delivery systems :

This type of delivery system relies on the strong peristaltic waves in the colon that leads to a temporarily increased luminal pressure. In the upper G.I.T., the drug delivery system is not directly subjected to the luminal pressure, since sufficient fluid is present in the stomach and small intestine. Due to raised luminal pressure in the colon, the system ruptures

and releases the drug. These strong peristaltic waves in the colon are of short duration, occurring only 3 to 4 times a day. The luminal pressure resulting from peristaltic motion is higher in the colon compared to pressure in the small intestine, which is attributed to the difference in the viscosity of luminal contents. In the stomach and small intestine, contents are fluidic because of abundant water in digestive juices, whereas in the colon, the viscosity of the content is significantly increased due to reabsorption of water from the lumen and formation of faeces. It has therefore concluded that drug dissolution in the colon could present a problem in relation to colon-specific oral drug delivery systems.

Pressure controlled colon delivery capsules were prepared using ethylcellulose, which is insoluble in water. In such systems, drug release occurs following the disintegration of a water-insoluble polymer capsule by the pressure in the lumen of the colon. The thickness of the ethylcellulose membrane is the most important factor for the disintegration of the formulation. The system also appeared to depend on capsule size and density. Because of reabsorption of water from the colon, the viscosity of luminal content is higher in the colon than in the small intestine. It has therefore been concluded that drug dissolution in the colon could present a problem in relation to colon-specific oral drug delivery systems. Lag time of 3 to 5 hours before drug absorption were noted when pressure controlled capsules were administered to humans.

2.2. Novel colon targeted delivery system (CODES^{TM}) :

CODES^{TM} is an unique CSDDS technology that was designed to avoid the inherent problems associated with pH or time dependent systems.CODES^{TM} is a combined approach of pH dependent and microbially triggered CSDDS. It has been developed by utilizing a unique mechanism involving lactulose, which acts as a trigger for site-specific drug release in the colon (Fig-1.13). The system consists of a traditional tablet core containing lactulose, which is over coated with an acid soluble material eudragit E and then subsequently overcoated with an enteric coating material eudragit L. The premise of the technology is that the enteric coating protects the tablet while it is located in the stomach and then dissolves quickly following gastric emptying. The acid soluble coating protects the preparation when it passes through the alkaline pH of the small intestine. Once the tablet arrives to the colon, the bacteria enzymatically degrade the polysaccharide (lactulose) into organic acid. This lowers the pH surrounding the system sufficient to effect the dissolution of the acid soluble coating and subsequent drug release.

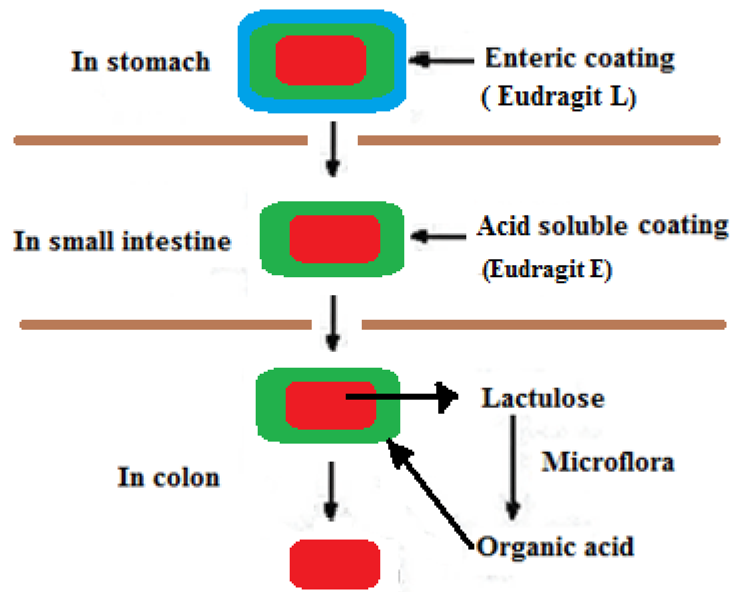

Fig-1.13. : Schematics of conceptual design of CODES™

2.3. Osmotic controlled drug delivery :

This approach is based on the principle of osmotic pressure. The osmotically controlled drug delivery system (Fig-1.14) can be used to target the drug locally or systemically. There are basically two types of formulations that operate on the principle of osmotic pressure. One is Osmet pump and another is OROS-CT.

Osmet pump is basically composed of enteric coated semi-permeable rigid shell, possessing an osmotic layer along with central impermeable and collapsible reservoir filled with drug molecules. The water penetrates through the semipermeable membrane and it leads to increase in pressure inside the device.

The OROS-CT (Alza corporation) can be used to target the drug locally to the colon for the treatment of disease or to achieve systemic absorption that is otherwise unattainable. The OROS-CT system can be a single osmotic unit or may incorporate as many as 5 to 6 push-pull units, each 4 mm in diameter, encapsulated within a hard gelatin capsule (Fig-1.15). Each bilayer push-pull unit contains an osmotic push layer and a drug layer, both surrounded by a semi-permeable membrane. An orifice is drilled through the membrane next to the drug layer. Immediately after the OROS-CT is swallowed, the gelatin capsule containing the push-pull units dissolves. Because of its drug-impermeable enteric coating, each push-pull unit is prevented from absorbing water in the acidic aqueous environment of the stomach, and hence no drug is delivered. As the unit enters the small intestine, the coating

dissolves in this higher pH environment (pH≥7), water enters into the unit causing the osmotic push compartment to swell and concomitantly creates a flowable gel in the drug compartment. Swelling of the osmotic push compartment forces drug gel out of the orifice at a rate precisely controlled by the rate of water transport through the semipermeable membrane. For treating ulcerative colitis, each push-pull unit is designed with a 3 to 4 hours post gastric delay to prevent drug delivery in the small intestine. Drug release begins when the unit reaches to the colon. OROS-CT units can maintain a constant release rate for up to 24 hours in the colon or can deliver drug over a period as short as 4 hours. Recently, new phase transit systems have come which promise to be a good tool for targeting drugs to the colon.

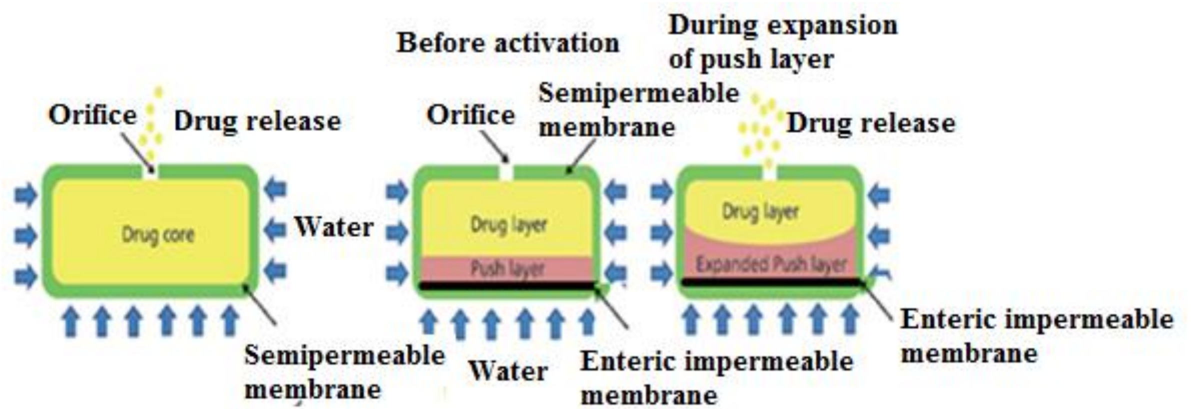

Fig-1.14. : <u>Osmotically controlled drug delivery system</u>

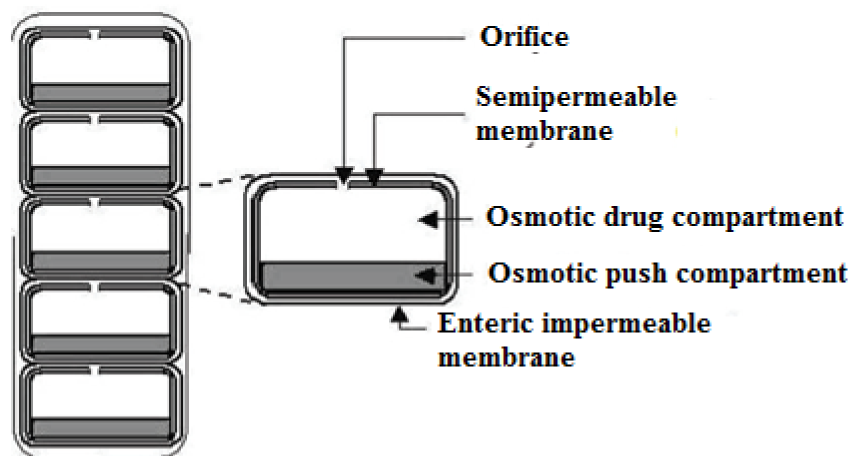

Fig-1.15. : <u>Cross section of the OROS-CT CSDDS</u>

2.4. <u>Hydrogel based drug delivery system</u> :

Three-dimensional cross-linked polymeric networks, also known as hydrogels, specially based on natural polysaccharides, have attracted a considerable attention as an excellent candidate for controlled release as well as targeted release devices. Hydrogels can absorb water many times their own weight and the release of drugs from hydrogels was determined by their swelling properties. The swelling extent is based upon the composition and pH of the surrounding medium. The swelling of hydrogel increases at higher pH values, which leads to increase in amount of drug release. In a study, a cross-linked three-dimensional network of Psyllium with acrylamide has been synthesized using potassium persulphate-hexamethylene tetramine as an initiator-crosslinker system. At lower pH, $-CONH_2$ group remains ionized and keeps the polymeric network in its collapsed state. But at higher pH, the partially ionized molecule leads to formation of repulsive COO^- groups, which increases the swelling extent resulting in more drug release (Fig-1.16).

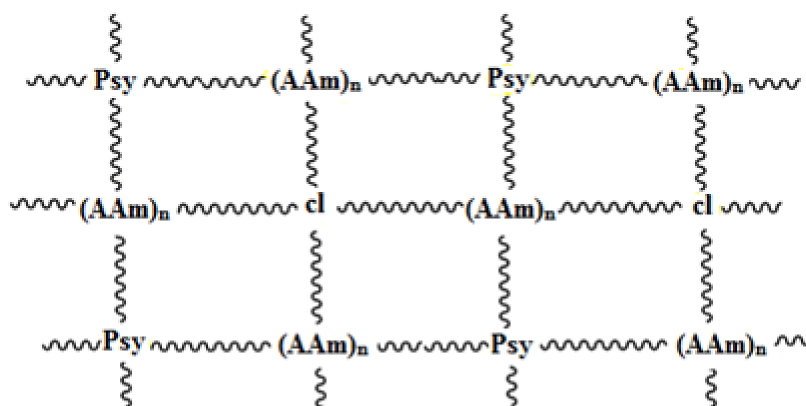

Fig-1.16. : <u>Inter-penetrating network of Psy-cl-poly(AAm)where cl=cross-linker ; $(AAm)_n$=polyacrylamide chain ; Psy=Psyllium</u>

2.5. <u>Compression-coating</u> :

Compression-coating tablet is another novel approach for delivery of drug to the colon (Fig-1.17). The compression-coating tablet contains an immediate release core tablet and a compression-coat over the core tablet. The compression-coating around the core tablet is made in such a way that it is not degraded in the upper G.I.T. and can pass the small intestinal transit time. After reaching to the colon, it delivers the drug in the colonic fluid (pH 6.8).

Advantages of such system are :

- It is industrially feasible to manufacture using the existing tablet manufacturing tools.
- No special coating solvent or coating equipment are needed for coating process.
- The release of the drug from the core tablet can be modulated to produce time dependent relese.
- If a suitable coating is used in the outer coating, it is possible to make enteric compression coated tablet.
- This dosage form makes it feasible to mask the unpleasant taste of drug.
- Repeat action could be produced by including drug in coating as well as in the core tablet to achieve pulsatile action.

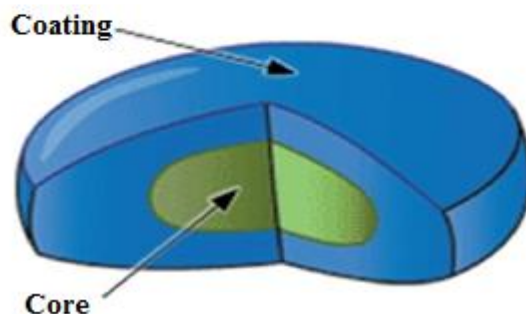

Fig-1.17. : Inner core and outer coat of a compression-coating tablet

PART II
LITERATURE REVIEW

During the last few decades, interest in the formulation of delayed release dosage forms such as colon-specific drug delivery systems have gained increasing importance for the treatment of various colonic diseases, such as amoebiasis, ulcerative colitis, Crohn's disease and colorectal cancer. Different strategies have been used for targeting drugs to the colon. The conventional approaches give rise to premature drug release. The combination of chemically modified forms of polysaccharides eliminated the drawbacks associated with the use of single polysaccharide. Among the various polysaccharides Locust bean gum (LBG) has been extensively investigated as polymeric material for developing tablets for colon targeted drug delivery systems. Extensive reports are available on the development of tablets as the delayed release drug delivery systems with the use of natural polymers as retarding material, as the polymers are safe, non-toxic, biodegradable and biocompatible.

- **Deshkar et. al., 2009,** developed colon targeted drug delivery system for ketoprofen using natural polysaccharides such as guar gum and locust bean gum as carrier. Matrix and compression-coated tablets of ketoprofen were prepared using both the gums. All the formulations were evaluated for the hardness, % drug content and subjected to in-vitro drug release studies. The amount of ketoprofen released from tablets at different time interval was estimated by UV-visible spectrophotometer. Guar gum and locust bean gum matrix tablets released about 60% and 80% of the ketoprofen, respectively, within 5 hours of the dissolution study and failed to control the drug release in the physiological environment of stomach and small intestine. The tablets, compression-coated with 300 mg of guar gum and locust bean gum released about 10% and 4% of ketoprofen respectively within 5 hours of the dissolution study. When the dissolution study was further continued in presence of rat caecal content medium, there was increase in release of ketoprofen due to biodegradation of gum coat. The results of the study showed that compression-coated ketoprofen tablets with 300 mg of guar gum coat are most likely to provide targeting of ketoprofen for local action in the colon owing to its minimal release in the first 5 hours.

- **Jenita et. al., 2010,** developed a colon-specific drug delivery system based on a natural polysaccharide; locust bean gum (550, 450, 350 and 250 mg) and are evaluated by in-vitro and in-vivo methods. The in-vitro study in pH 6.8 phosphate buffer, containing 4% w/v rat caecal contents, showed the cumulative % release of mesalazine after 26 hours as 33.75%±0.1988, 46.25%±0.9640 and 95.75%±0.1013 respectively.

These studies on the polysaccharide indicated that locust bean gum as a coating material, proved to be capable of protecting the mesalazine core tablet under conditions mimicking mouth to colon transit. The in-vivo studies conducted in nine healthy human volunteers for the various formulations revealed that the drug release was initiated only after 5 hours i.e. transit time of stomach, small intestine and the bioavailability (AUC_{0-t}) of the drug was found to be 147.985±0.5, 249.57±0.17, 480.075±0.069 μg h/ml respectively. This study clearly established that locust bean gum in the form of compression coat is a potential carrier for drug targeting to the colon.

- **Chickpetty & Raga, 2010,** developed colon targeted compression-coated delivery systems for diclofenac sodium by using different proportion of guar gum (GG) and locust bean gum (LBG) mixture in the ratio 1:1 in combination with hydroxypropylmethyl cellulose (HPMC) as a coating material. Effect of proportion of GG-LBG mixture:HPMC ratio on % of drug release in upper G.I.T. and in the colon was studied on developed formulations. It was found that compression-coated formulations released 0-6.70% of diclofenac sodium in the physiological environment of stomach and small intestine. The compression-coated formulations containing GG-LBG mixture:HPMC in the ratios of 9:1, 8:2, 7:3, 6:4 and 5:5 released 35.84%, 47.62%, 78.61%, 94.82% and 98.03% of diclofenac sodium respectively in simulated colonic fluid indicating the susceptibility of gum mixture to the rat caecal contents. The results revealed that the tablets compression-coated with GG-LBG mixture and HPMC in the ratio 6:4 is most likely to provide targeting of diclofenac sodium for local action in the colon owing to their minimal drug release in physiological environment of stomach, and small intestine and more than 90% of drug release in the colon. The IR study indicates that the drug is intact in the formulation and no possibility of interaction between the diclofenac sodium and guar gum or locust bean gum or other formulation excipients exist.

- **Navneet & Pooja, 2011,** developed a successive colon targeted delivery of compression-coated aceclofenac tablets. The influence of locust bean gum and xanthan gum polymers and their various combinations was studied on drug release profile. The preformulation studies like FT-IR spectroscopy and differential scanning calorimetry (DSC) showed the absence of drug-excipient interactions. The tablets

were found within the permissible limits for various physicochemical parameters. Dissolution studies were performed in 0.1 N HCl for 2 hours, in pH 7.4 buffer for 3 hours and the in pH 6.8 buffer up to 24 hours. The in-vitro studies were also performed in pH 6.8 phosphate buffer containing 4% w/v rat caecal content. The cumulative % release of aceclofenac after 24 hours was found to be 61.40±1.02%, 54.43±1.86%, 46.27±1.96%, 39.37±2.70%, 49.63±2.88% (mean±S.D.), for formulation LX1, LX2, LX3, LX4, LX5 respectively. The effect of presence of rat ceacal content (4% w/v) on cumulative % drug release was observed significantly positive. The formulations containing locust bean gum alone showed rapid release of drug whereas combination of locust bean gum and xanthan gum was found to be sufficient to sustain the drug release for successful colon targeting. Present study on the polysaccharides demonstrated that the combination of locust bean gum and xanthan gum as a coating material proved capable of protecting the core tablet containing aceclofenac during the condition mimicking mouth to colon transit.

- **Chickpetty et. al., 2012,** developed aceclofenac compression-coated tablets by using locust bean gum and guar gum mixture in the ratio 1:1 along with xanthan gum as coating carrier materials. The core tablet containing 100 mg of aceclofenac were compression coated with a coat weight of 400 mg containing gum (LBG:GG) mixture and XG in the ratio 6:1, 5:2, 4:3 and 3:4. The developed coated tablets were evaluated for hardness, friability, weight variation and content uniformity. Drug release studies were carried out in pH 1.2 for 2 hours, pH 7.4 for 3 hours and then in pH 6.8 up to 24 hours with or without rat caecal content to mimic the physiological conditions from the mouth to colon. The formulation released 0-4.72% of drug in first 5 hours and released 33.19-52.97% of the drug at the end of 24 hours in control study. The increase in drug release was observed from all the formulations in presence of 4% w/v rat caecal content indicating susceptibility of gum mixture to the rat caecal content. The in-vitro release studies revealed that, gum mixture and HPMC in the ratio 5:2 were found to be suitable for targeting aceclofenac to the colon without being released significantly in upper G.I.T. FT-IR studies indicated no drug-excipient interactions.

- **Kaluri et. al., 2012,** developed some formulation of aceclofenac with mixture of gum (Locust bean gum, Xanthan gum, Guar gum, starch paste). The quantity of LBG,GG,XG and starch paste present in the formulation code LGX61

(150,150,50,40), LGX52 (125,125,100,40), LGX43 (100,100,150,40) and LGX34 (75,75,200,40) respectively. The % drug released from the formulations LGX61, LGX52, LGX43 and LGX34 was found to be 5.18%, 1.22%, 1.12% and 0%, respectively, in the initial 5 hours of dissolution study in simulated gastric (2 hours) and intestinal fluids (3 hours). The drug release in first 5 hours decreased as the proportion of XG content in the coat increases. To assess the integrity of coat, the drug release studies were continued up to 24 hours without the addition of rat caecal content to dissolution medium. At the end of 24 hours of dissolution studies, the mean % drug release from LGX61, LGX52, LGX43 and LGX34 was 52.95%, 43.25%, 38.56%, and 33.19%. To assess the ability of the coat to release the drug in physiological environment of colon, the in-vitro drug release studies were carried out in pH 6.8 phosphate buffer saline solution containing rat caecal contents. When the dissolution studies were carried out in the presence of 4% w/v rat ceacal content medium, the mean % drug release from coat formulation LGX61 and LGX52 was found to be 86.27% and 96.13%, respectively at the end of 24 hours. The study showed that as the proportion of XG content increases in the coat formulations LGX43 and LGX34, the drug release was decreased significantly in the physiological environment of colon.

- **Dey et. al., 2012**, developed and evaluated carboxymethyl locust Bean Gum-Al3^{+} alginate hydrogel network for gastrointestinal delivery of glipizide. Sodium alginate and carboxymethyl locust bean gum (CMLBG) were reticulated in an aqueous solution of $AlCl_3$ and this novel inter-penetrating network (IPN) hydrogel encapsulated about 93-98% glipizide. An increase in the CMLBG weight ratio and the degree of cross-linking in the IPN was found to increase mean dissolution time of the encapsulated drug. The dissolution efficiency was found to be much higher in the medium at pH 7.4 than at pH 1.2. The swelling of IPN depended on the pH of the medium, and accordingly, monitored the drug release for a period of 8 hours. The anomalous drug transport mechanism was presumed to be operative. High performance liquid chromatography (HPLC) analysis showed the drug’s stability in the IPN during encapsulation. The IPN showed significant hypoglycemic activity on male wistar rats for up to 10 hours.

- **Bashardoust et. al., 2013,** developed compression-coated tablets of ibuprofen minimizing drug release in the upper G.I.T. and targeting the colon using the principles of compression-coating. Ibuprofen tablets were prepared by a direct compression method using locust bean gum (LBG) at 300, 250, 200 and 175 mg and evaluated for their physicochemical properties and in-vitro drug release. In-vitro drug release studies were performed with and without rat caecal content. In rat caecal content, tablets showed enhanced drug release due to degradation of the LBG coating by colonic enzymes. The in-vitro release studies in pH 6.8 phosphate buffer containing 2% w/v rat caecal content showed the cumulative % release of ibuprofen after 26 hours as 39.91±0.05%, 53.21±0.37%, 69.17±0.19% and 94.46±0.92%. Coating thickness and the amount of chitosan control the release rate. Formulations were best fitted with Korsmeyer–Peppas kinetics, and the mechanism of drug release was non-Fickian super case II transport. FT-IR studies reveal there is no drug–polysaccharide interaction. The F1 formulation is a promising system for drug targeting to the colon.

- **Sidramappa & Baswaraj, 2013,** developed 5-fluorouracil compression-coated tablets by using biodegradable polysaccharide locust bean gum (LBG) and hydroxypropylmethyl cellulose (HPMC) as coating material. The fast disintegrating core tablets containing 50 mg of 5-fluorouracil were compression-coated with LBG and HPMC in different ratios (8:1, 7:2 and 6:3) with a coat weight of 300, 400 and 500 mg. In-vitro dissolution data indicated that the formulation (CLH63) with a coat weight of 500 mg containing LBG and HPMC in the ratio 6:3 gave the best release profile (0% in first 5 hours and 96.18% in 24 hours). DSC and FT-IR results indicated no possibility of interaction between drug and polymers or other excipients. In-vivo human X-ray studies revealed that formulation CLH63 was able to resist breakdown in the stomach and small intestine. The disintegration of the tablet occurred in the colon between 8 to 16 hours of post dose. By the present study, it can be concluded that the LBG and HPMC based compression-coated tablets of 5-fluorouracil will be useful strategy for colonic delivery of 5-fluorouracil without being released in upper G.I.T. for the safe and effective management of colon cancer.

The literature survey indicates that the use of natural polysaccharides either alone or in combination with other natural polymers may lead to a drug delivery systems that delay the drug release. Such delivery systems are capable of reducing premature drug release in the

upper G.I.T. and providing release of drugs in colon. Several combination of LBG with other natural polysaccharides have been examined. However there is no report on the development of colon targeted tablets using a blend of LBG and its carboxymethylated derivative CMLBG as compression coating material. Thus the objective of this study was to develop compression-coated tablet of MNZ using several blends of LBG and its semi-synthetic derivative CMLBG.

PART III
AIM &
OBJECTIVE

3.1. Aim and objective :

Colon, the terminal part of G.I.T. is susceptible to various diseases like Crohn's disease, ulcerative colitis, Irritable bowel syndrome, diverticular disease, colorectal polyps, colon cancer and various colonic infections. Rational therapy of these diseases require attainment of sufficient concentration of drugs in the colon preventing the premature drug release in the upper G.I.T.

As it is the distal part of G.I.T., the conventional oral dosage forms of drugs fail to reach the colonic region because of variations in pH along the entire G.I.T., various enzymatic degradations and versatile movement of food materials in the tract.

In recent years, a large number of solid dosage formulations targeting the lower parts of the G.I.T., especially the colon, have been reported to achieve the therapeutic activity of drug. The various approaches used for targeting the drugs to the colon include formation of a prodrug, multicoating, time dependent delivery systems, coating with pH-sensitive polymers, pressure dependent systems and the use of biodegradable polymers.

pH dependent (or delayed release) systems are designed to release a drug in response to change in pH usually above a particular pH of the G.I.T. But these systems have poor site specificity because of large variations in the pH in the entire G.I.T., variations in gastric emptying time and passage across the ileocaecal junction (12).

Time dependent (or time release) systems are designed to release a drug after a predetermined time period of administration (38,39).

Pressure dependent systems make use of luminal pressure of the colon.

Microbially dependent (or microbially trigerred) systems use the abundant enterobacteria present in the colon (40). It involves the use of natural polysaccharides that are degraded exclusively by colonic bacteria (13). The microflora of colon is in the range of 10^{11}-10^{12} CFU/ml consisting mainly of anaerobic bacteria, e.g. bacteroides, bifidobacteria, eubacteria, clostridia, enterococci, enterobacteria and ruminococcus (41). This enormous microflora present in the colon fulfils their energy need by fermenting various types of substrates that have been left undigested in the small intestine, e.g. di- and tri-saccharides, polysaccharides etc. (42) and produce a huge number of enzymes like β-glucoronidase, β-xylosidase, α-arabinosidase, β-galactosidase, nitro-reductase, azoreducatase, deaminase and

urea dehydroxylase (43,44), which help to cleave the backbones of biodegradable polymers used for colonic drug delivery.

Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be a more site-specific approach with good colonical activity as compared to other approaches. These polymers shield the drug from the environments of stomach and small intestine and are able to deliver the drug in sufficiently high concentration to the colon. On reaching the colon they undergo assimilation by the micro-organisms or degradation by enzymes or break down of the polymeric backbones, leading to subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer (43,45). Therefore the use of natural biodegradable polymer is the most suitable approach for colon targeting. Still they have some limitations like considerable variations in content of colonic bacteria with age, diseased state, antibiotic therapy and food habits. So the aim of our work extends to development of a polysachharide based colon targeting drug delivery system that is independent of the action of colonic microfloral enzyme.

Locust bean gum (LBG), obtained from the endosperms of seeds of *Ceratonia siliqua* family Leguminosae, is a nonionic, natural polysaccharide composed of galactomannans and consists of 1,4-linked β-D-mannopyranose backbone with branch points from their 6 positions linked to single α-D-galactose residue (1,6-linked α-D-galactopyranose).

LBG exists as poorly water soluble “random coils” whereas dissolved LBG adopts a disordered, fluctuating random coil conformation. Galactomannans need high temperature and vigorous agitation for complete dissolution in water. To improve the water solubility and viscosity profile and to impart gelling ability, it requires chemical modification like carboxymethylation (46). LBG contains numerous functional groups. These groups can be chemically modified into carboxylated, hydroxylated and phosphate derivatives of galactomannans to impart newer properties to the parent gum e.g. better water binding capacity and increased water solubility.

Various polysaccharides have been used as coating materials because of their natural degradation by the colonic microflora. Although LBG alone or with other polysachharides has been utilized to develop sustained or delayed release coated tablets (47) yet the native gum with combination of its derivative have not been explored as a compression-coating material to develop pulsatile release tablets with above mentioned release criteria. Hydration of gum

seems not to be affected by pH 3 to 11 of dissolution medium protecting release in stomach, small intestine. It also remains stable against variations in salinity and temperature. Moreover, biodegradability of the gum by colonic bacterial enzymes (Fig-3.1) proves locust bean gum as a suitable carrier for colonic drug delivery system. Any swellable, erodible, hydrophilic, biodegradable polymer can be used along with native gum to improve release which was declined due to formation of swollen thick viscous gel layer when native gum was used alone.

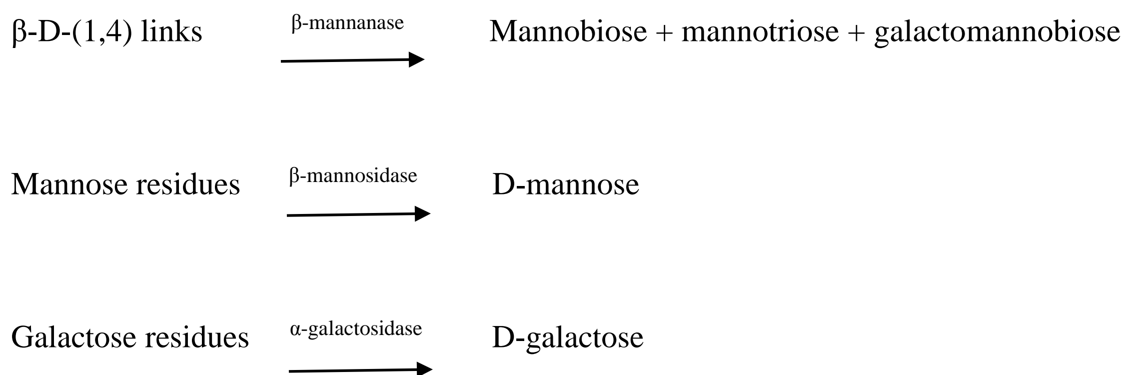

Fig- 3.1. : Degradation of Locust bean gum

Compression-coated tablet is another novel approach for delivery of drug to the colon. It contains a core tablet having drug incorporated into it and a compression-coat over the core tablet (48). Modern compression-coated tablets are made on specialized equipment in two separate operations : manufacture of core tablets on a traditional tablet press and the subsequent application of a compression-coating granulation. A compression-coating is expected to protect the drug release during the transit in the G.I.T. and to allow its release only in the colon. The coating of core tablet is made in such a way that it is not degraded by lower pH of the acidic environment in stomach or enzymes present in small intestine. After reaching to colon it delivers the core tablet at the colonic pH 7 to 8 and the core tablet being an immediate release dosage form, releases the drug instantly into the colon.

The objective of the present work was to assess the suitability of a blend of LBG and CMLBG (the carboxymethyl derivative) as compression-coating material for developing a compression-coated tablet for colon delivery of drugs. Although LBG is susceptible to degradation with colonic microflora, objective was to formulate tablets in such a way that it can deliver the drugs even in the absence of colonic microflora. Release of metronidazole

(MNZ), a model drug, was evaluated from the compression-coated tablets prepared with LBG and CMLBG blended coating.

3.2. Drug rationality :

Metronidazole (MNZ) is the prototype nitroimidazole. It is a highly active broad spectrum amoebicide used particularly against anaerobic bacteria and protozoa. Many anaerobic bacteria such as *Bacteroids fragilis*, *Clostridium perfringens, Clostridium difficile, Helicobactor pylori,* campylobacter, fusobacterium, peptococci, spirochetes and anaerobic streptococci are sensitive. It is the drug of choice for a first episode of mild-to-moderate *Clostridium difficile* colitis. It is a first line drug for all forms of amoebic infection. It is used for both intestinal and extraintestinal amoebiasis. It is used either alone or in combination with other antibiotics such as ceftriaxone, to treat pelvic inflammatory disease, giardiasis, bacterial vaginosis, enterocolitis, endocarditis, gingivitis and gastritis. It is also effective for dracunculiasis, trichomoniasis, aspiration pneumonia, rosacea (topical), fungating wounds (topical), intra-abdominal infections, lung abscess and peridonitis.

So MNZ is the most preferred drug for the treatment of intestinal amoebiasis (49). This drug is to be delivered to the colon for effective action against E. histolytica, wherein the trophozoites reside in the lumen of the caecum, large intestine and adhere to the colonic mucus and epithelial layers (50).

PART IV
MATERIALS

4.1. Metronidazole :

- Metronidazole was supplied as sample gift by the CAPLET India Pvt. Ltd, Kolkata India.

4.2. Locust Bean Gum :

- Purchased from Himedia Laboratory Pvt. Ltd. Mumbai.

4.3. Sodium hydroxide (NaOH) :

- Mol Wt. : 40
- Assay : 97%
- Laboratory reagent grade
- Finar, chemicals Ltd. Ahmedabad-380006.

4.4. Hydrochloric Acid (HCl) :

- Mol Wt. : 36.46
- Assay : 35 to 38%
- Sp.Gravity : 1.18
- Merck Specialities Pvt. Ltd. Shiv sagar estate "A" Dr. Annie Besant Road. Worli, Mumbai-400018.

4.5. Methanol :

- Purity : 99%
- Analytical reagent grade
- Merck Specialities Pvt. Ltd. Shiv sagar estate "A" Dr. Annie Besant Road. Worli, Mumbai-400018.

4.6. Glacial acetic acid :

- Assay : 99.8%
- Thermo Fisher Scientific India Pvt. Ltd. Mumbai-400022.

4.7. Phenolphthalein :

- Qualigens fine chemica. Dr. Annie Besant Road Bombay-400025.

4.8. Monochloroacetic Acid :

- Assay : 99.0%
- S.D fine-chem Ltd. Mumbai-400025.

4.9. Silicon dioxide :

- Gift smple by Caplet India Pvt.Ltd.

PART V
DRUG PROFILE

5.1. General Information :

- **Name :** Metronidazole
- **Chemical Formula :** $C_6H_9N_3O_3$
- **Molecular weight :** 171.15 g/ml
- **Chemical structure :**

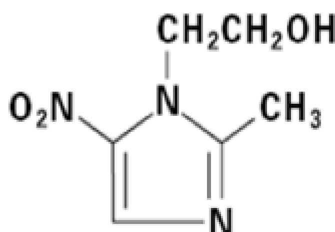

Fig-2.1. : Metronidazole

- **Chemical name (IUPAC) :** 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol
- **Category :** Anti-microbial, anti-amoebic
- **Storage :** Store in well closed, air tight container

5.2. Physical properties :

- **Description :** A white crystalline powder
- **Solubility :** Metronidazole solubility in water was reported as 10 mg/ml at 20°C and 10.5 mg/ml at 25°C
- **M.P :** 159 to 163°C (318 to 325°F)
- **pKa :** MNZ is a basic compound with pKa value 2.62

5.3. Mechanism of action :

Metronidazole (Fig-2.1) is the prototype nitroimidazole highly active broad spectrum amoebicide used particularly against anaerobic bacteria and protozoa (3). Some anaerobic protozoal parasites (including amoeba) possess ferrodoxin like electron transport proteins that participate in metabolic electron removal reactions. The nitro group of MNZ is able to serve as an electron acceptor, forming reduced cytotoxic compound, highly active nitro radical that bind to proteins and DNA, causes DNA helix destabilization and strand breakage, resulting in

cell death. Many anaerobic bacteria such as *Bacteroides fragilis*, fusobacterium, *Clostridium perfringens, Clostridium difficile*, *Helicobactor pylori,* campylobacter, peptococci, spirochets and anaerobic streptococci are sensitive.

5.4. Pharmacokinetics :

MNZ is almost completely absorbed from the small intestine, little unabsorbed drug reaches to the colon.The pharmacokinetic properties of MNZ and its five major metabolites have been investigated intensively. The hydroxy metabolite has biological activity of 30 to 65% and a longer elimination half-life than the parent compound. Oral, intravenous, intravaginal and topical preparartion of MNZ are available. MNZ given orally is absorbed almost completely, with bioavailability more than 90% for tablets, absorption is unaffected by infection. Rectal and intravaginal absorption are 67 to 82% and 20 to 56% of the dose, respectively. Plasma concentration reaches to 8 to 13 µg/ml within 0.25 to 4 hours after administration of a single 500 mg dose. Mean effective concentration of the compound is less than 8 µg/ml for most susceptible protozoa and bacteria. A linear relationship between dose and plasma concentration has been found for doses of 200 to 2000 mg. Repeated doses after every 6 to 8 hours result in some accumulation of the drug. Systemic clearance exhibits dose dependence. The $t_{1/2}$ of MNZ in plasma is 8 hours, its volume of distribution approximates total body water. Less than 20% of the drug is bound to plasma proteins. With the exception of the placenta, MNZ penetrates well into body tissues and fluids including vaginal secretions, semen, saliva, breast milk and CSF. After an oral dose, more than 75% of labelled MNZ is eliminated in the urine largely as metabolites where 10% is recovered as unchanged drug. The liver is the main site of metabolism, and this accounts for more than 50% of the systemic clearance of MNZ. The two principal metabolites result from oxidation of side chains area hydroxyl derivative and an acid. The hydroxyl metabolite has a longer $t_{1/2}$ (12 hours) and has 50% of the anti-trichomonal activity of that of MNZ. Formation of glucuronides is also observed. Small quantities of reduced metabolites, including ring cleavage products, are formed by the gut flora. The urine of some patients may be reddish brown owing to the presence of unidentified pigments derived from the drug.

5.5. Therapeutic uses and dosage :

1. Amoebiasis :

MNZ is a first line drug for all forms of amoebic infections. Many dosage regimens have been tried. the current recommendations are for invasive dysentery and liver abscess : 800 mg TDS (children 30 to 50 mg/kg/day) for 7 to 10 days. In serious cases of liver abscess : 1 g may be infused through i.v. slowly, followed by 0.5 g every 8 to 12 hours till oral therapy is instituted. For mild intestinal disease : 400 mg TDS for 5 to 7 days is recommended. MNZ is less effective than many luminal amoebicides in eradicating amoebic cysts from the colon, because it is nearly completely absorbed from the upper bowel.

2. Giardiasis :

MNZ is highly effective in a dose of 400 mg TDS for 7 days. A shorter course of 3 days with 2 g/day is equally effective.

3. Trichomonas vaginitis :

MNZ is the drug of choice, 400 mg TDS for 7 days achieves nearly 100% cure. Additional intravaginal treatment has been given, but is not necessary except in refractory cases. The male partner should be treated concurrently in cases of recurrent infections. Non-specific bacterial vaginosis also responds.

4. Anaerobic bacterial infections :

They occur mostly after colorectal or pelvic surgery, appendicectomy etc. Brain abscesses and endocarditis may be caused by anaerobic organisms.

5. Pseudo membranous enterocolitis :

It is caused by Cl. difficile. Oral MNZ 800 mg TDS is more effective, more convenient, less toxic and therefore preferred over vancomycin.

6. Ulcerative gingivitis, trench mouth :

200-400 mg TDS (15 to 30 mg/kg/day) is quite effective because anaerobes are involved. MNZ or tinidazole are the drugs of choice for acute necrotizing ulcerative gingivitis, in which they are often combined with amoxicillin, tetracycline or erythromycin. The response is rapid with disappearance of the spirochete-fusobacterium complex from the

lesions and resolution of pain, bleeding, ulceration and bad breath within 2 to 3 days, but treatment must be continued for at least 5 days.

7. *Helicobacter pylori* gastritis or peptic ulcer :

MNZ alone are relatively ineffective in eradicating H. pylori, if resistance develops. However, MNZ 400 mg TDS is frequently used along with amoxicillin or clarithromycin and a proton pump inhibitor as triple drug 2 week regimens.

8. Guinea worm infestation :

MNZ is used in India because of non availability of niridazole. A 7 day course with 200 to 400 mg TDS produces symptomatic relief. The local reaction to the worms may be suppressed by its anti-inflammatory action and extraction is facilitated.

5.6. Side effects, contraindications and drug interactions :

Side effects are frequent but only rarely severe enough to discontinue therapy.

- The most common are anorexia, nausea and a metallic taste.
- Vomiting, diarrhoea and abdominal cramp, looseness of stool are experienced occasionally.
- Less frequent side effects are headache, glossitis, dryness of mouth, dizziness, rashes and transient neutropenia.
- Furry tongue and stomatitis occurring during therapy may be associated with an exacerbation of candidiasis.
- Prolonged administration may cause peripheral neuropathy and CNS effects.
- Seizures have followed very high doses.
- Vertigo and very rarely encephalopathy, convulsions, incoordination and ataxia are neurotoxic effects that warrant discontinuation of MNZ.
- The drug also should be withdrawn if numbness or paresthesias of the extremities occur.
- Urticaria, flushing, and pruritus are indicative of drug sensitivity that can require withdrawal of MNZ.
- Dysuria, cystitis and a sense of pelvic pressure have been reported.
- Thromboflebitis of injected vein occurs if the solution is not well diluted.

Contraindications of MNZ include :

- Patients suffering from liver disease, a stomach or intestinal disease such as Crohn's disease, a blood cell disorder such as anemia (lack of red blood cells) or leukopenia (lack of white blood cells), epilepsy or other seizure disorder; nerve disorders may need a dose adjustment or special tests to safely take this medication.
- MNZ should be used with caution in patients with active disease of the CNS because of its potential neurotoxicity.
- Mutagenic activity is associated with MNZ and several of its metabolites found in the urine of patients treated with therapeutic doses of the drug. Due to its potential carcinogenic properties, MNZ is banned in the European Union and the USA for veterinary use in the feed of animals and is banned for use in any food materials in the USA.
- MNZ has been taken during all stages of pregnancy with no apparent adverse effects, but its use during the first trimester and during lactation generally is not advised because although there is no evidence that therapeutic doses of MNZ pose any significant increased risk of cancer to human patients but there is conflicting evidence about the teratogenicity of metronidazole in animals.
- Patients should be cautioned to avoid consuming alcohol during MNZ treatment.
- The drug also may precipitate CNS signs of lithium toxicity decreasing renal elimination of lithium in patients receiving high doses of lithium.
- MNZ can prolong the prothrombin time of patients receiving therapy with coumarin anticoagulants.
- The dosage of MNZ should be reduced in patients with severe hepatic disease.

MNZ shows interactions with many drugs such as :

- It has a well documented disulfiram like effect and some patients experience abdominal distress, vomiting, flushing or headache if they drink alcoholic beverages during or within 3 days of therapy with this drug. Patients should be cautioned to avoid consuming alcohol during MNZ treatment even though the risk of a severe reaction is low. So MNZ and disulfiram or any disulfiram like drug should not be taken together because confusional and psychotic states may occur. Although related chemicals have caused blood dyscrasias, only a

temporary neutropenia, reversible after discontinuation of therapy, occurs with MNZ.

- Oxidative metabolism of MNZ is induced by enzyme inducers e.g. phenobarbital, prednisolone, rifampin and possibly ethanol.
- Cimetidine appears to inhibit hepatic metabolism of the drug.
- The drug also may precipitate CNS signs of lithium toxicity decreasing renal elimination of lithium in patients receiving high doses of lithium.
- Moreover, MNZ can prolong the prothrombin time of patients receiving therapy with coumarin anticoagulants.
- MNZ alone rarely causes Stevens–Johnson syndrome, but is reported to occur at high rates when combined with mebendazole.

PART VI
METHODOLOGY

6.1. Preparation of acid and phosphate buffer solutions :

6.1.1. Preparation of 0.1 (N) acid solution of pH 1.2 :

8.5 ml of concentrated hydrochloric acid (HCl) was taken in a volumetric flask containing 200 ml distilled water and the volume was then made up to 1000 ml with distilled water and finally pH was adjusted to 1.2, using a pH meter (model Orion 2-star, Thermo Scientific).

6.1.2. Preparation of phosphate buffer (PB) solution of pH 7.4 and pH 6.8 :

For the preparation of phosphate buffer solution of pH 7.4, 700 ml of HCl solution (pH 1.2) was prepared by the process mentioned above and then 200 ml 2 (M) Tri-sodium Orthophosphate Dodecahydrate solution was added to make the pH 7.4. To prepare a solution of pH 6.8, 2 (M) 5 ml HCl solution was added to the phosphate buffer solution of pH 7.4. In each case the pH of the solution was adjusted with HCl or tri-sodium orthophosphate solution and checked with the pH meter.

6.2. Determination of wavelength of maximum absorbance (λmax) of metronidazole :

6.2.1. Determination of wavelength of maximum absorbance (λmax) of metronidazole in acid solution of pH 1.2 :

25 mg of drug was dissolved in acid solution of pH 1.2 and volume was made upto 500 ml. Then 4 ml of the above solution was further diluted to 25 ml with acid solution and the solution was scanned from 200 to 800 nm in UV-VIS spectrophotometer (Shimadzu UV 2450, Japan). The spectrum has been shown (Fig-6.1) and the wavelength of maximum absorbance was found at 278 nm.

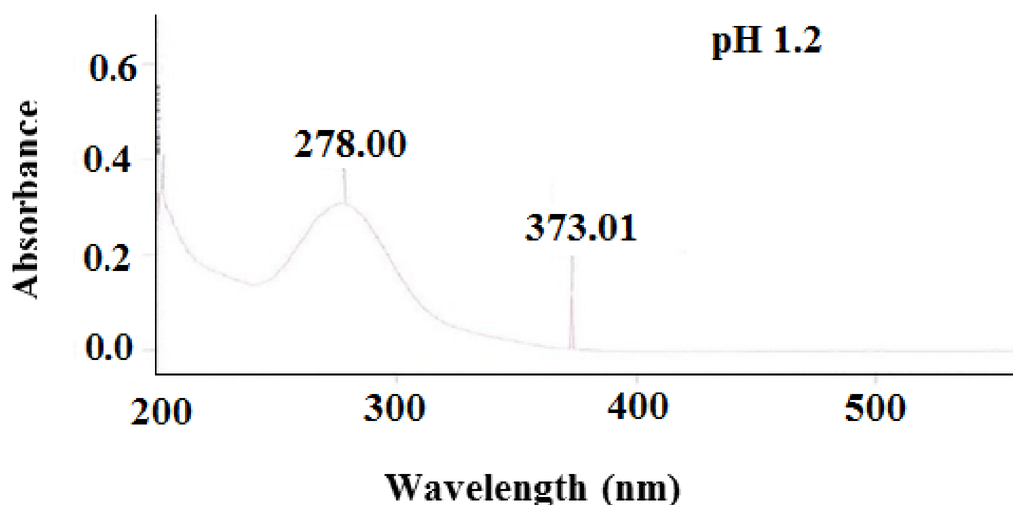

Fig-6.1. : UV spectrum of metronidazole in acid solution of pH 1.2

6.2.2. Determination of wavelength of maximum absorbance (λmax) of metronidazole in phosphate buffer solution of pH 6.8 :

25 mg of drug was dissolved in phosphate buffer solution of pH 6.8 and volume was made upto 500 ml. Then 4 ml of the above solution was further diluted to 25 ml with buffer solution and the solution was scanned from 200 to 800 nm in UV-VIS spectrophotometer (Shimadzu UV 2450, Japan). The spectrum has been shown (Fig-6.2) and the wavelength of maximum absorbance was found at 320 nm.

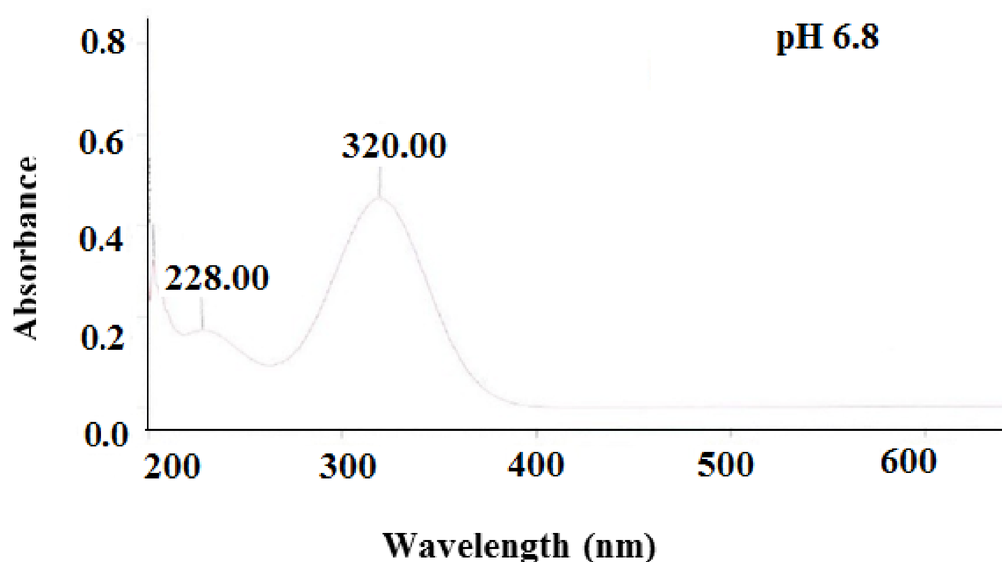

Fig-6.2. : UV spectrum of metronidazole in phosphate buffer solution of pH6.8

6.2.3. Determination of wavelength of maximum absorbance (λmax) of metronidazole in phosphate buffer solution of pH 7.4 :

25 mg of drug was dissolved in phosphate buffer solution of pH 7.4 and volume was made upto 500 ml. Then 4 ml of the above solution was further diluted to 25 ml with buffer solution and the solution was scanned from 200 to 800 nm in UV-VIS spectrophotometer (Shimadzu UV 2450,Japan). The spectrum has been shown (Fig-6.3) and the wavelength of maximum absorbance was found at 320 nm.

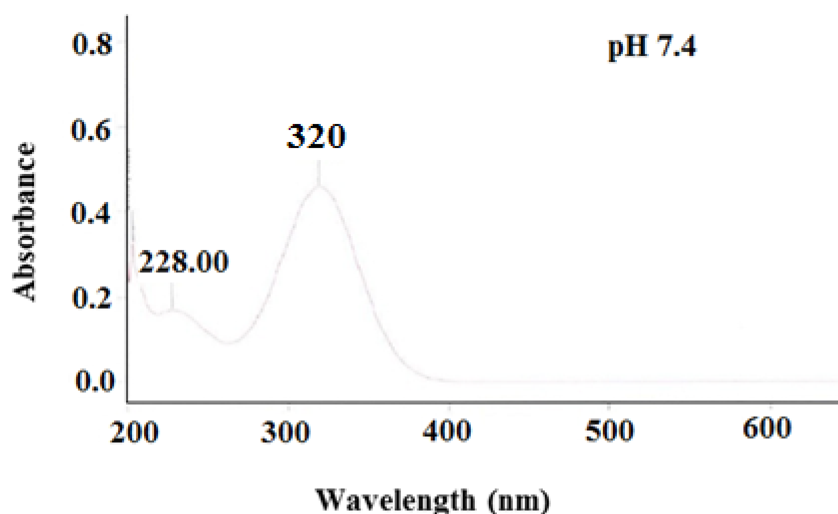

Fig-6.3. : UV spectrum of metronidazole in phosphate buffer solution of pH 7.4

6.3. Calibration curves of metronidazole :

6.3.1. Calibration curve of metronidazole in acid solution of pH 1.2 :

Calibration curve of MNZ in acid solution was drawn spectrophotometrically at 278 nm. 500 mg of MNZ was weighed accurately (Precisa Electronic Balance, model XB 600M/C, Switzerland) and dissolved in acid solution and the volume was made up to the 500 ml with the acid solution. From this, 1 ml solution was drawn and diluted to 100 ml with acid solution. From this stock solution, 1,2,4,6,8 and 10 ml (concentration 0 to 20 µg/ml) was diluted to 25 ml with the acid solution. Absorbance of these solutions were determined at 278 nm (Table-6.1). The experiment was conducted six times and average values were used to

draw the calibration curve. The calibration curve (Fig-6.4) was linear upto 20 µg/ml with a correlation coefficient (R^2) 0.9996.

Table-6.1. : Calibration curve data of metronidazole in acid solution of pH 1.2 :

Concentration of metronidazole (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Absorbance IV	Absorbance V	Absorbance VI	Average absorbance	Mean ±SD (n=6)
2	0.11	0.09	0.10	0.09	0.09	0.09	0.09	0.10± 0.008
4	0.18	0.18	0.18	0.18	0.19	0.17	0.18	0.18± 0.006
8	0.38	0.35	0.36	0.36	0.36	0.36	0.36	0.36± 0.010
12	0.56	0.54	0.55	0.51	0.53	0.52	0.53	0.54± 0.019
16	0.75	0.73	0.73	0.71	0.73	0.72	0.72	0.73± 0.013
20	0.91	0.88	0.88	0.88	0.90	0.88	0.88	0.89± 0.013

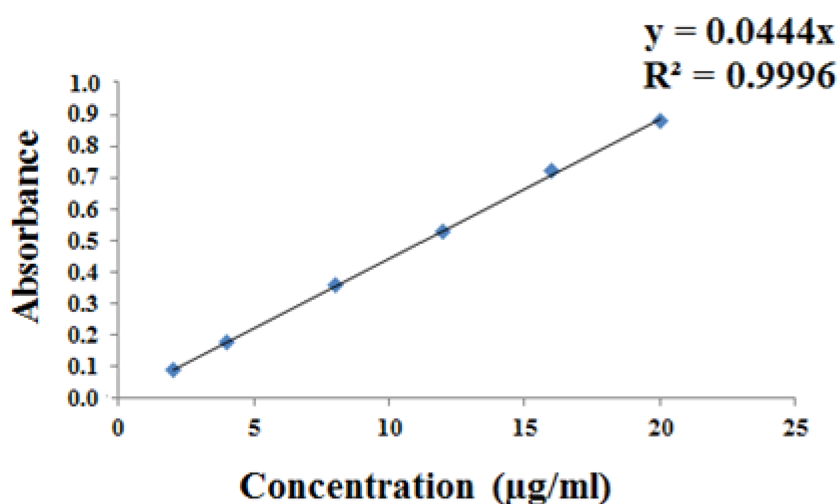

Fig-6.4. : Calibration curve of metronidazole in acid solution of pH 1.2

6.3.2. Calibration curve of metronidazole in phosphate buffer solution of pH 6.8 :

Calibration curve of MNZ in phosphate buffer solution of pH 6.8 was drawn spectrophotometrically at 320 nm. 500 mg of MNZ was weighed accurately (Precisa Electronic Balance, model XB 600M/C, Switzerland) and dissolved in phosphate buffer solution and the volume was made up to the 500 ml with the buffer solution. From this, 1 ml solution was drawn and diluted to 100 ml phosphate buffer solution. From this stock solution 1,2,4,6,8 and 10 ml (concentration 0 to 20 µg/ml) was diluted to 25 ml with the phosphate buffer solution (pH 6.8). Absorbance of these solutions were determined at 320 nm (Table-6.2). The experiment was conducted six times and average values were used to draw the calibration curve. The calibration curve (Fig-6.5) was linear upto 20 µg/ml with a correlation coefficient (R^2) 0.9998.

Table-6.2. : Calibration curve data of metronidazole in phosphate buffer solution of pH 6.8 :

Concentration of metronidazole (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Absorbance IV	Absorbance V	Absorbance VI	Average absorbance	Mean ±SD (n=6)
2	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12± 0.000
4	0.22	0.23	0.22	0.22	0.23	0.24	0.23	0.23± 0.008
8	0.48	0.46	0.47	0.46	0.47	0.47	0.47	0.47± 0.008
12	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72± 0.000
16	0.94	0.94	0.93	0.93	0.94	0.96	0.94	0.94± 0.011
20	1.18	1.18	1.18	1.18	1.18	1.20	1.18	1.18± 0.008

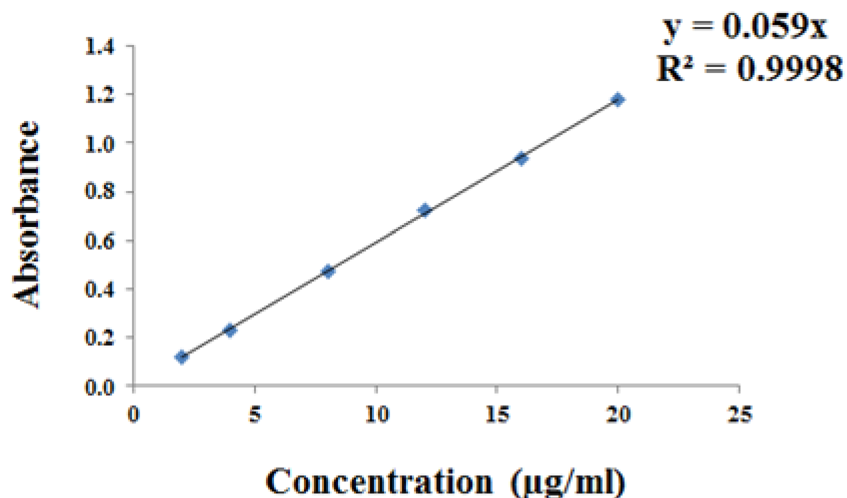

Fig-6.5. : Calibration curve of metronidazole in phosphate buffer solution of pH 6.8

6.3.3. Calibration curve of metronidazole in phosphate buffer solution of pH 7.4 :

Calibration curve of MNZ in phosphate buffer solution (pH 7.4) was drawn spectrophotometrically at 320 nm. 500 mg of MNZ was weighed accurately (Precisa Electronic Balance, model XB 600M/C, Switzerland) and dissolved in phosphate buffer solution and the volume was made up to the 500 ml with the solution. From this, 1 ml solution was drawn and diluted to 100 ml phosphate buffer solution. From this stock solution 1,2,4,6,8 and 10 ml (concentration 0 to 20 µg/ml) was diluted to 25 ml with the phosphate buffer solution (pH 7.4). Absorbance of these solutions was determined at 320 nm (Table-6.3). The experiment was conducted six times and average values were used to draw the calibration curve. The calibration curve (Fig-6.6) was linear upto 20 µg/ml with a correlation coefficient (R^2) 0.9999.

Table-6.3. : Calibration curve data of metronidazole in phosphate buffer solution of pH 7.4 :

Concentration of metronidazole (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Absorbance IV	Absorbance V	Absorbance VI	Average absorbance	Mean ±SD (n=6)
2	0.13	0.12	0.13	1.12	0.12	0.13	0.12	0.13± 0.005

4	0.25	0.24	0.25	0.24	0.24	0.25	0.24	0.25± 0.005
8	0.50	0.48	0.50	0.48	0.48	0.51	0.49	0.49± 0.013
12	0.75	0.75	0.76	0.72	0.72	0.76	0.74	0.74± 0.019
16	1.01	0.99	1.01	0.98	0.98	1.01	0.99	1.00± 0.015
20	1.27	1.21	1.29	1.21	1.21	1.29	1.24	1.25± 0.041

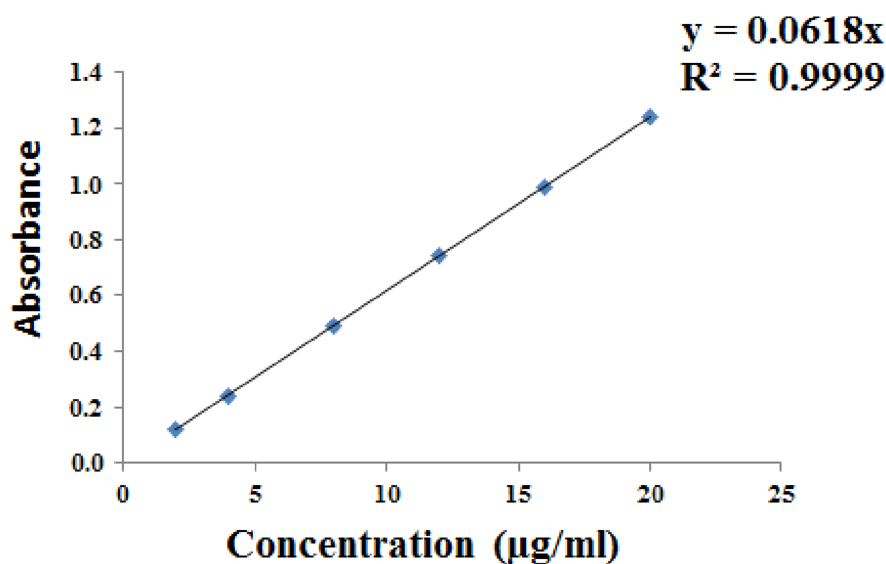

Fig-6.6. : Calibration curve of metronidazole in phosphate buffer solution of pH 7.4

6.4. Modification of locust bean gum :

Locust bean gum was modified to sodium carboxymethyl locust bean gum by a two step base catalyzed reaction procedure. 2 gm accurately weighed gum was slowly sprinkled, small quantity at a time, in ice cold deionized water (6.72 ml) containing 3.024 g of NaOH with vigorous stirring. The temperature of the reaction mixture was maintained at 0 to 8°C for 30 minutes. Monochloroacetic acid (1.5 g) dissolved in 3.33 ml of deionized water was added slowly for a period of 30 minutes and the temperature of the reaction mixture was maintained

at 15 to 18°C. The reaction mixture was transferred to a thermostatically controlled water bath and heated at 75°C for 1 hour with constant stirring. The wet mass was washed with 3 successive amounts of 20 ml 80% methanol for 15 minutes each. During last washing, the pH of the suspension was adjusted to neutrality with glacial acetic acid. Finally it was washed with pure methanol and kept for drying at 45 to 50°C until constant weight was obtained. The reaction mechanism for carboxymethylation has been shown schematically (Fig-6.7).

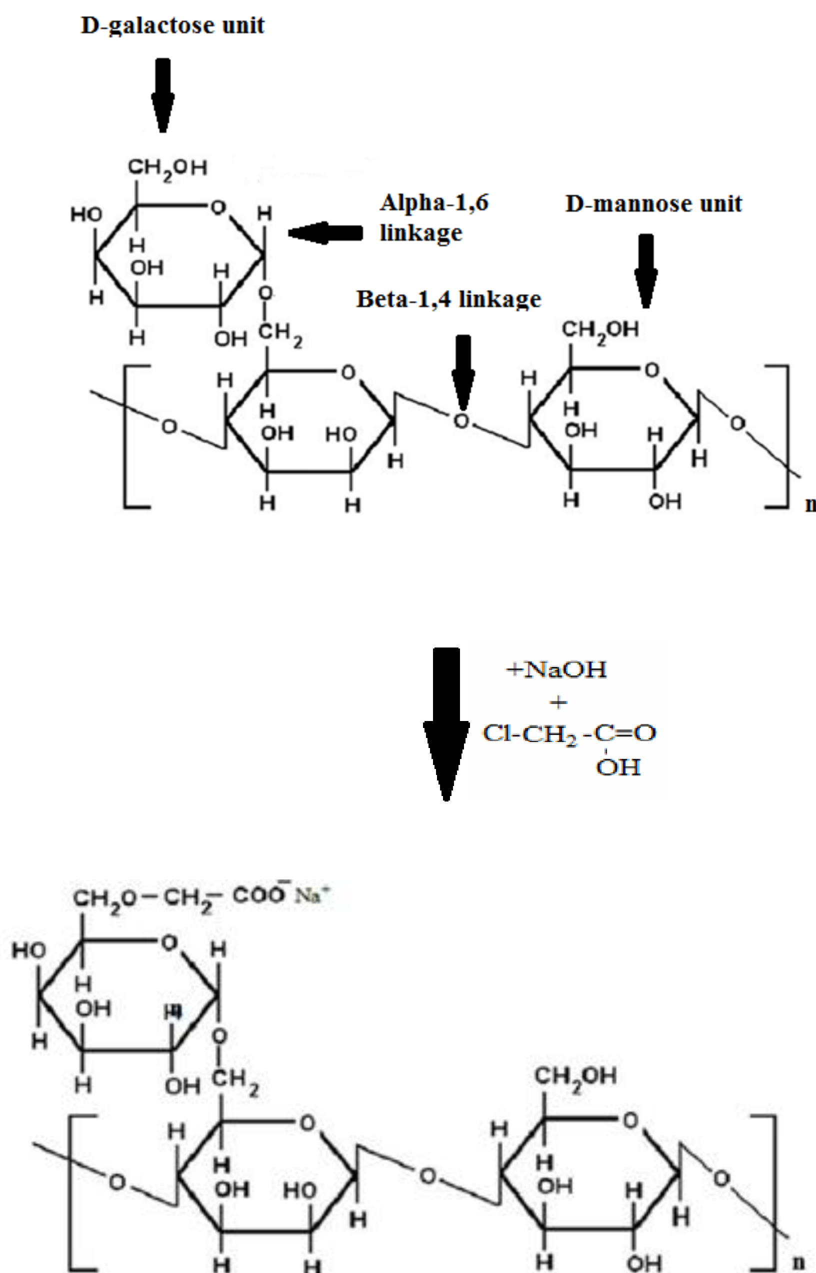

Fig-6.7. : Carboxymethylation of locust bean gum

6.5. Characterization of sodium carboxymethyl locust bean gum :

6.5.1. Determination of degree of substitution :

About 500 mg accurately weighed sodium carboxymethyl locust bean gum was dispersed in 5 ml 80% v/v methanol and concentrated hydrochloric acid was added in slight excess to convert the sodium form into hydrogen form and stirred for 2 to 3 hours. Then 3 to 4 ml methanol was added, mixed well and filtered through Whatmann filter paper (no.1). The residue was washed with 5 ml 80% methanol, until washing showed neutrality to litmus paper. Finally it was washed with pure methanol and dried. 200 mg accurately weighed dried gum was taken. 1.5 ml 70% v/v methanol was added and allowed to stand for few minutes. Then 20 ml water and 5 ml 0.5 (N) NaOH solution were accurately measured and added. The mixture was shaken for 3 to 4 hours until the sample dissolved. The solution was then back titrated with 0.4 (N) hydrochloric acid solution to phenolphthalein end point.

Degree of substitution (DS) was calculated using the following formula.

$DS = 0.162A/1-0.058A$

Where, A is milliequivalents of NaOH required per gram of sample.

6.5.2. Fourier transform infrared (FT-IR) analysis :

FT-IR spectra of finely powdered dried locust bean gum and sodium carboxymethyl locust bean gum were recorded in FT-IR spectrophotometer (IR-Prestige-21 Shimadzu, Japan). Each sample was mixed with KBr and converted into disc at 5.5 ton pressure using a hydraulic press. The spectra was taken in the wave number region of 4000 to 400 cm^{-1}.

6.6. Preparation of core tablets :

MNZ core tablets (having a weight of 150 mg) were prepared by classical wet granulation technique. Required amounts of drug (100 mg), microcrystalline cellulose (Avicel), magnessium stearate as lubricant, cross povidine as super disintegrant, Trisodium citrate as osmogen were blended manually for 15 minutes (Table-6.4).

Core tablets containing 10% w/w trisodium citrate were prepared. Appropriate amount of binder (3% w/v aqueous solution of HPMC E15) was added to prepare a cohesive mass. The cohesive mass thus obtained were passed through 18 mesh screen and the granules were

then dried in a tray dryer at 60°C for sufficient period of time so that the moisture content of the granules reached 2 to 4%. The dried granules were passed through 22 mesh screen and then dried again in tray dryer at 60°C. Finally the calculated amount of dried granules were punched using 6 mm flat faced punch in a 10 station rotary mini-press tablet machine (RIMEK, Karnavati Engineering Ltd., Gujarat, India).

Table-6.4. : Composition of a single core tablet :

Metronidazole (mg)	Avicel (mg)	Cross povidone (mg)	Magnessium stearate (mg)	Tri sodium citrate (mg)
100	27.5	6	1.5	15

6.7. Preparation of compression-coated tablets :

Locust bean gum and sodium carboxymethylated locust bean gum (synthesized and passed through sieve number 60 to obtain uniform sized particle) were blended manually for 15 minutes. Appropriate amount of water was added to prepare a cohesive mass. The cohesive mass thus obtained were passed through 18 mesh screen and the granules were then dried in a tray dryer at 60°C for sufficient period of time so that the moisture content of the granules reached 2 to 4%. The dried granules were passed through 22 mesh screen and then dried again in tray dryer at 60°C. 0.05% silicone dioxide was blended with the granules. Three different types of granules consisting of LBG and CMLBG in a ratio of 50:50, 30:70 and 10:90 were prepared. 40% of the dried granules were placed in a 10 mm die cavity and the core tablet was placed at the centre. Finally remaining 60% granules were placed over the core tablet and compression-coated with a 10 mm flat face punch in a 10 station rotary mini-press tablet machine (RIMEK, Karnavati Engineering Ltd, Gujarat, India). The weight of the compression-coating material was varied from 250 mg to 350 mg. The tablets coated with 250 mg, 300 mg and 350 mg were designated as CW1, CW2, CW3 respectively.

6.8. Evaluation of tablets :

6.8.1. Hardness test of core and compression-coated tablets :

Hardness of each tablet was measured using Monsanto hardness tester.

6.8.2. Weight variation test of core and compression-coated tablets :

20 tablets, collected at random, were weighted individually and average weight was determined. The variation of the weight of each tablet from the average weight was determined.

6.8.3. Thickness test of core and compression-coated tablets :

Thickness of each tablet was measured using a slide calliperse (Digimatic Calliper, model CD-6"CS, Mitutoyo Corporation, Japan). Average thickness of 10 tablets was reported.

6.8.4. Friability test of core and compression-coated tablets :

10 tablets were weighted and taken in a Roche friabilator (Friabilator, Veego, and Mumbai, India). The tablets were rotated at 25 rpm for 4 minutes. The tablets were then de-dusted and reweighed. Average friability of 10 tablets was calculated.

6.8.5. Content uniformity test of core tablets :

10 tablets were collected at random. Each tablet was powdered in a glass mortar. The powder was quantitatively transferred in a 500 ml conical flask and 250 ml acid solution of pH 1.2 was added. The stoppered flask was shaken for 2 hours in a mechanical shaker. The mixture was filtered and an aliquot following suitable dilution was analyzed spectrophotometrically at 278 nm for MNZ content and finally potency of the tablet was determined using the calibration curve constructed using acid solution.

6.8.6. Disintegration time test of core tablets :

6 core tablets were placed in six tubes of disintegrating apparatus containing 900 ml phosphate buffer solution of pH 6.8 and maintained at 37±2°C. The cylinders were allowed to move up and down at 29 **to** 32 cycles per minute.The time taken by the tablets to disintegrate completely into small particles was noted. Average disintegration time of 6 tablets was calculated.

6.8.7. In-vitro drug release study of compression-coated tablets :

In-vitro drug release studies were carried out in USP II tablet dissolution rate test apparatus (model TDP-06P, Electro Lab, Mumbai, India) at 37±0.5°C and 75 r.p.m speed and the methodology was adopted from the Indian Pharmacopoeia, 2010. In order to simulate the diverse pH regions of the G.I.T., continuous change in buffer system was used for the modified release compression-coated tablets. The dissolution study was carried out in acid solution of pH 1.2 mimicking stomach pH for first 2 hours, then in pH 7.4 phosphate buffer solution mimicking small intestinal pH for further 5 hours and finally in pH 6.8 phophate buffer solution mimicking colonic pH condition upto 12 hours. 10 ml aliquot was withdrawn from the dissolution medium at predetermined time points and replaced with 10 ml of fresh respective fluid. The absorbance was measured at the respective wavelength of maximum absorbance for acid solution and buffer solutions of pH 7.4 and pH 6.8 using double beam spectrophotometer (Shimadzu UV 2450,Japan). The amount of drug released from the tablet was determined using calibration curves drawn in the respective medium. Each experiment was done in triplicate.

PART VII
RESULTS

7.1. Characterization of carboxymethyl locust bean gum (CMLBG) :

7.1.1. Determination of degree of substitution of O-carboxymethyl group in CMLBG :

The determination of degree of substitution of carboxymethyl locust bean gum was conducted by the method described in 6.5.1 and the results are shown in Table-7.1 & Table-7.2.

Table-7.1. : Determination of Degree of substitution of CMLBG :

No. of observations	Weight of sodium carboxymethylated locust bean gum (mg)	Volume of 80% v/v methanol (ml)	Volume of water (ml)	Volume of sodium hydroxide (ml)
1	200	1.5	20	5.0
2	200	1.5	20	5.0
3	200	1.5	20	5.0

Table-7.2. : Determination of Degree of Substitution of CMLBG :

Strength of sodium hydroxide (N)	Total volume of mixture (ml)	Volume of 0.4 (N) Hydrochloric acid used (ml)	A	Degree of substitution	Mean
0.5	26.50	6.0	3.25	0.65	0.67
0.5	26.50	6.2	3.60	0.72	
0.5	26.50	6.0	3.25	0.65	

- **Calculation :**

Degree of substitution is defined as the number of functional groups per unit residue of polymer chain that can be substituted by chemical modification. A milliequivalent of sodium hydroxide required per gram of sodium carboxymethyl locust bean gum (A) was calculated as follows :

Strength of HCl solution after standardization with standardized NaOH solution is 0.33 (N) following the equation ($V_1S_1=V_2S_2$) of alkalimetry and acidimetry at neutrality,

0.33 (N)×6=26.5×(x) (N) = 0.07 (N)

26.5 ml of 0.07 (N) NaOH = 1.85 meq of NaOH and

5.0 ml of 0.5(N) NaOH = 2.5 meq of NaOH

Therefore, consumed NaOH corresponds to 0.65 meq.

Thus, A=(0.65×1000)/200=3.25 meq of NaOH per gram of sodiumcarboxymethyl locust bean gum.

The average DS value was found to be 0.67.

The values of "A" were calculated similarly for rest of the two occasions.

7.1.2. FT-IR study of LBG and CMLBG :

FT-IR spectra of LBG and CMLBG are represented in Fig-7.1. The broad band of -OH stretching vibrations appeared at 3442.3 cm^{-1} (46) and 3300.2 cm^{-1} (34). Stretching is attributed to -OH group of LBG. But in case of CMLBG, it was broaden. This might be an indication of the utilization of hydroxyl groups in the carboxymethylation reaction. The C-H stretching vibrations of $-CH_2$ were observed at 2931.80 cm^{-1} (51). The bands due to ring stretching of galactose and mannose appeared at 1649.14 cm^{-1}. The presence of carboxymethylated groups in CMLBG were confirmed by the peaks at about 1327.03 cm^{-1} that indicated the carboxymethylation of -C=O group of LBG.

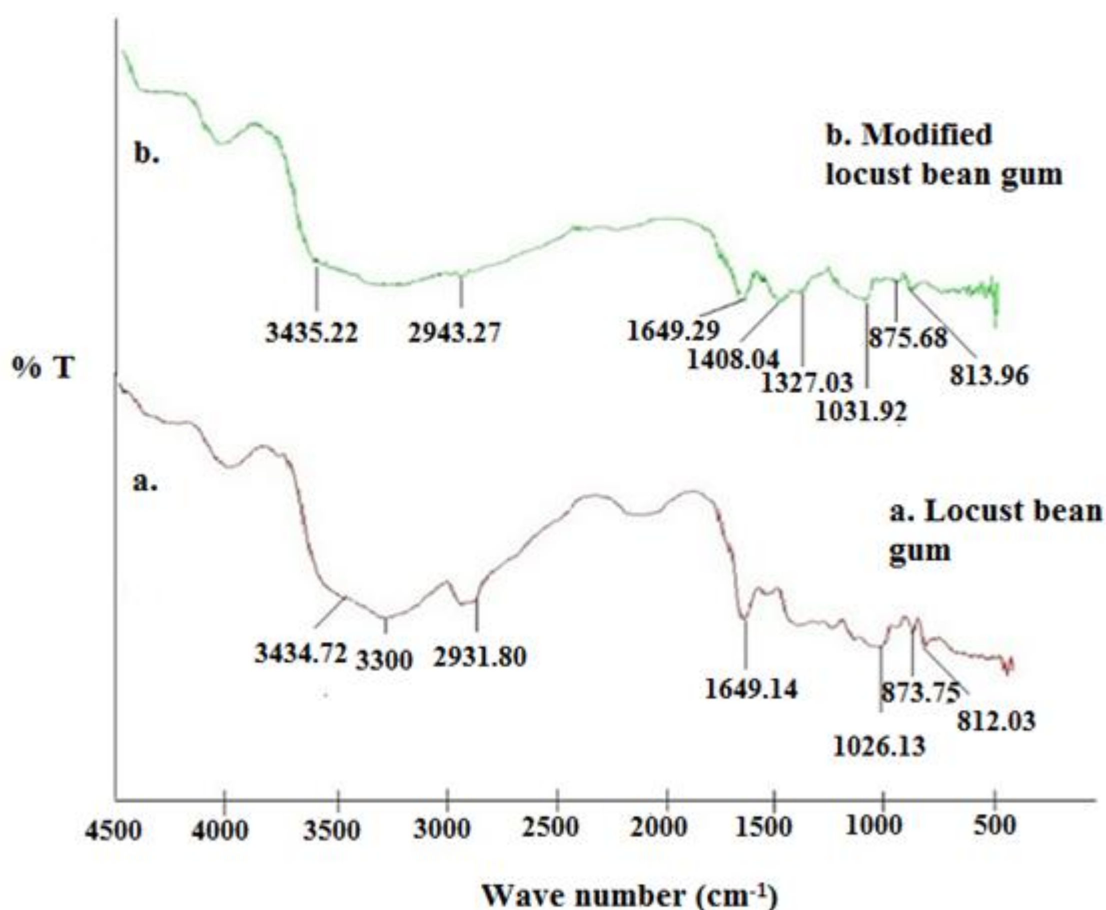

Fig-7.1. : <u>FT-IR spectra of LBG and CMLBG</u>

7.1.3. <u>Drug-polymer compatibility in compression-coated tablets</u> :

The compatibility of the drug in compression-coated tablets was assessed through FT-IR analysis. FT-IR spectra of MNZ showed the characteristics bands of -OH stretching, C-H & C=CH stretching, N-O stretching, $-NO_2$ symmetrical stretching and -C-O stretching respectively at 3223.05, 3101.54, 1537.27, 1369.46 and 1074.35 cm^{-1}. The spectra obtained from the powdered tablet (Fig-7.2) showed the presence of the all above characteristic bands of the drug almost at the same wave numbers indicating compatibility of drug with polymer.

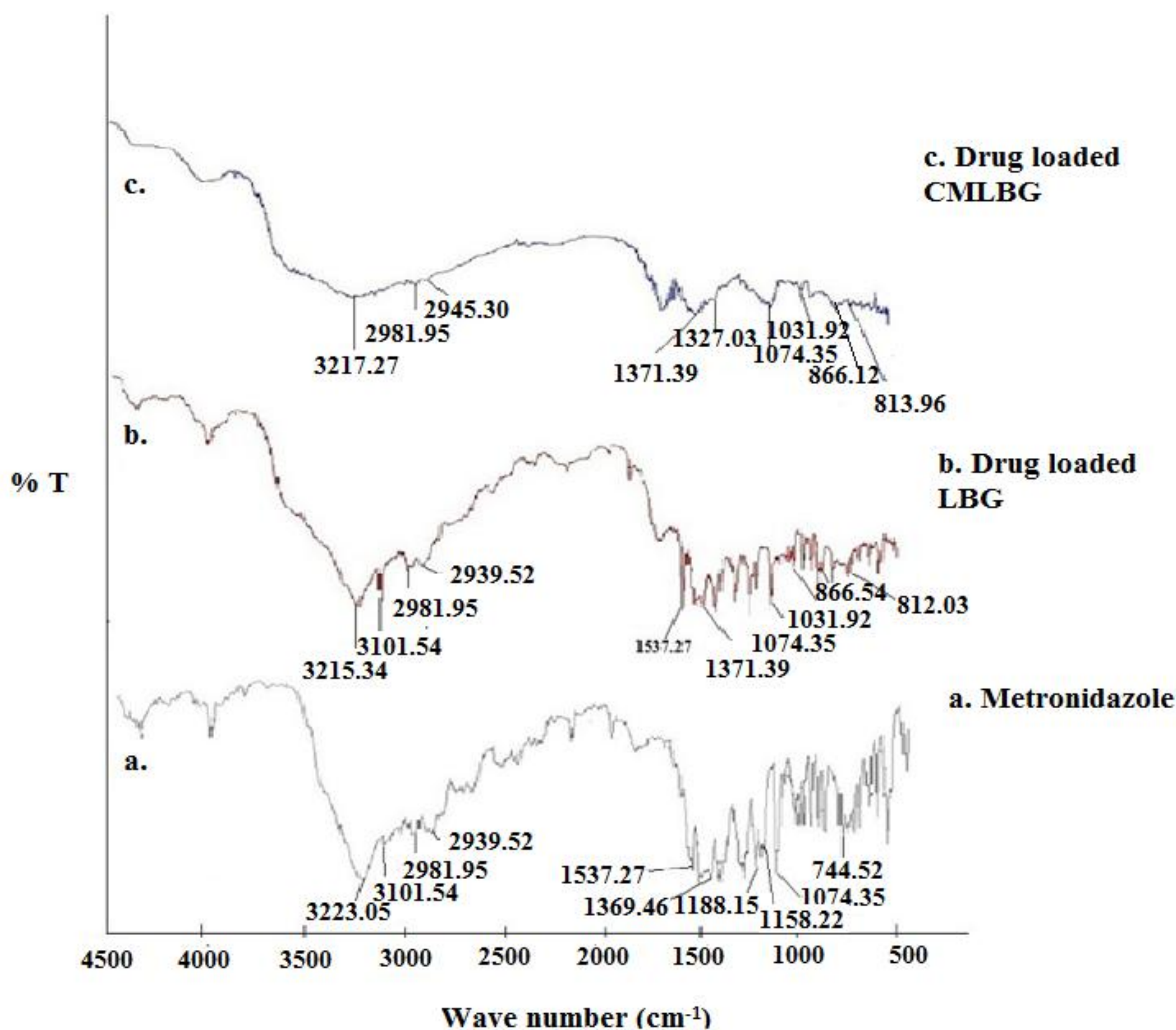

Fig-7.2. : <u>FT-IR spectra of a. metronidazole b. drug loaded LBG and c. drug loaded CMLBG compression-coated tablet</u>

7.2. Physical characteristics of tablets :

7.2.1. Hardness :

Table-7.3. : Hardness of core tablets containing 10% w/w osmogen :

Batch No.	Hardness (kg)	Average hardness (kg)±S.D (n=10)
1	1.5	1.75±0.26
2	1.5	
3	1.5	

4	2.0	
5	1.5	
6	2.0	
7	2.0	
8	2.0	
9	1.5	
10	2.0	

Table-7.4. : Hardness of compression-coated tablets with core tablet containing 10% w/w osmogen (Hardness in kg±S.D where n=10) :

Coat weight	**Average hardness of tablets compression-coated with different ratios of LBG and CMLBG**		
	50:50	**30:70**	**10:90**
250 mg	2.5±0.43 kg	3±0.43 kg	4±0.56 kg
300 mg	3.5±0.52 kg	4±0.31 kg	6±0.38 kg
350 mg	4.5±0.40 kg	5±0.48 kg	6.5±0.49 kg

7.2.2. Weight variation :

Table 7.5. : Weight variation of core tablets containing 10% w/w osmogen :

Batch No.	**Weight (g)**	**Average weight (g)±S.D (n=20)**
1	149.5	149.21±0.59
2	148.9	
3	149.3	
4	150.2	
5	148.6	
6	149.9	
7	148.2	
8	149.7	
9	149.5	
10	148.7	

11	149.3	
12	149.8	
13	148.2	
14	148.9	
15	148.4	
16	149.7	
17	149.3	
18	148.7	
19	149.8	
20	149.5	

Table 7.6. : Weight variation of compression-coated tablets with core tablet containing 10% w/w osmogen (Weight variation in g±S.D where n=20) :

Coat weight	**Average weight of tablets compression-coated with different ratios of LBG and CMLBG**		
	50:50	**30:70**	**10:90**
250 mg	400.18±0.21	402.18±0.11	401.18±0.31
300 mg	451.23±0.32	452.23±0.22	452.23±0.12
350 mg	501.23±0.34	500.23±0.14	501.23±0.24

7.2.3. Thickness :

Table 7.7. : Thickness of core tablets containing 10% w/w osmogen :

Batch No.	**Thickness (cm)**	**Average thickness (cm)±S.D (n=10)**
1	2.25	2.25±0.006
2	2.24	
3	2.25	
4	2.25	
5	2.25	
6	2.26	
7	2.24	

8	2.24	
9	2.25	
10	2.25	

Table 7.8. : Thickness of compression-coated tablets with core tablet containing 10% w/w osmogen (Thickness in cm±S.D where n=10) :

Coat weight	**Average thickness of tablets compression-coated with different ratios of LBG and CMLBG**		
	50:50	**30:70**	**10:90**
250 mg	3.1±0.013	3.0±0.012	3.0±0.013
300 mg	3.3±0.015	3.3±0.009	3.2±0.014
350 mg	3.5±0.016	3.4±0.005	3.3±0.017

7.2.4. Friability :

Table 7.9. : Friability of core tablets containing 10% w/w osmogen :

Batch No.	**% Friability**	**Average % Friability±S.D (n=10)**
1	0.24	0.48±0.13
2	0.56	
3	0.43	
4	0.52	
5	0.62	
6	0.46	
7	0.67	
8	0.49	
9	0.34	
10	0.45	

Table 7.10. : Friability of compression-coated tablets with core tablet containing 10% w/w osmogen (% Friability±S.D where n=10) :

Coat weight	Average friability of tablets compression-coated with different ratios of LBG and CMLBG		
	50:50	30:70	10:90
250 mg	0.51±0.035	0.26±0.029	0.48±0.043
300 mg	0.48±0.051	0.35±0.069	0.50±0.058
350 mg	0.35±0.060	0.59±0.065	0.62±0.046

7.2.5. Drug content :

Table 7.11. : Drug content of core tablets containing 10% w/w osmogen :

Batch No.	Actual drug content (mg)	Experimental drug content (mg)	Average experimental drug content (mg)±S.D (n=10)
1	100	99.8	99.7±0.11
2	100	99.6	
3	100	99.6	
4	100	99.8	
5	100	99.7	
6	100	99.5	
7	100	99.8	
8	100	99.6	
9	100	99.7	
10	100	99.8	

7.2.6. Disintegration :

Table 7.12. : Disintegration of core tablets containing 10% w/w osmogen :

Batch No.	Disintegration time (minutes)	Average disintegration time (minutes) ±S.D (n=6)
1	2	1.67±0.51
2	1	
3	2	
4	2	
5	1	
6	2	

7.3. Drug release study :

Table 7.13. : Cumulative % drug release from metronidazole compression-coated tablets containing 10% w/w osmogen in core and LBG:CMLBG=50:50 in coat in different amounts :

Time (min)	250 mg (CW1)		300 mg (CW2)		350 mg (CW3)	
	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
15	0.31±0.13	2.33±1.01	0.23±0.00	1.75±0.00	0.23±0.00	1.75±0.00
30	0.40±0.14	5.28±1.76	0.31±0.13	4.10±1.01	0.24±0.00	3.52±0.00
45	0.55±0.14	7.10±1.79	0.32±0.14	4.75±2.03	0.24±0.00	3.57±0.00
60	0.72±0.24	9.53±1.72	0.40±0.14	5.39±1.79	0.24±0.00	3.62±0.00
75	0.80±0.14	11.41±2.76	0.41±0.14	6.05±2.06	0.25±0.00	3.67±0.00
90	1.05±0.14	13.90±2.15	0.57±0.13	7.30±1.79	0.25±0.00	3.72±0.00
105	1.14±0.28	16.43±3.19	0.65±0.27	9.05±3.04	0.33±0.13	4.35±1.01
120	1.31±0.37	18.40±4.82	0.74±0.24	10.44±3.72	0.34±0.14	5.00±2.03

150	1.17±0.20	37.16±8.51	0.77±0.19	22.63±6.49	0.33±0.11	9.96±3.18
180	1.18±0.20	35.14±5.98	0.78±0.19	23.20±5.77	0.39±0.18	10.81±4.16
210	1.31±0.23	37.30±6.30	0.79±0.20	23.44±5.83	0.40±0.18	11.81±5.46
240	1.74±0.38	45.77±8.98	1.09±0.39	28.17±8.72	0.52±0.28	13.73±6.92
270	2.06±0.24	57.03±9.07	1.40±0.43	37.45±12.27	0.94±0.55	21.96±11.47
300	2.14±0.21	63.02±6.55	1.78±0.29	47.73±10.77	1.13±0.37	31.19±13.83
330	2.46±0.29	69.06±6.92	1.86±0.23	54.53±7.79	1.33±0.20	36.91±8.58
360	2.73±0.37	77.86±9.85	2.00±0.30	57.79±7.98	1.34±0.20	40.00±5.98
390	2.94±0.37	84.95±11.19	2.26±0.41	63.79±10.66	1.47±0.23	42.21±6.20
420	3.15±0.31	91.22±10.00	2.40±0.31	69.84±10.77	1.67±0.30	47.15±7.47
450	4.27±0.38	111.25±8.39	3.28±0.51	85.17±12.28	2.23±0.50	58.43±11.91
480	5.06±0.63	140.04±14.81	3.76±0.51	105.61±15.31	2.40±0.48	69.38±14.53
510	6.92±0.72	179.70±20.11	5.15±0.96	133.68±22.04	3.47±0.97	88.08±21.66
540	9.09±0.84	240.02±23.50	6.70±0.97	177.78±28.61	4.56±0.73	120.48±25.59
570	12.18±0.73	318.96±22.15	8.35±1.42	225.74±35.84	5.58±1.07	152.09±27.01
600	13.58±0.75	386.40±19.78	10.68±1.95	285.44±50.41	6.99±0.97	188.55±30.46
660	17.17±1.14	922.57±55.32	13.19±1.23	716.28±94.58	9.46±1.88	493.47±85.18
720	18.03±0.84	1055.93±55.29	14.53±0.83	831.77±61.11	11.06±1.67	615.60±105.26

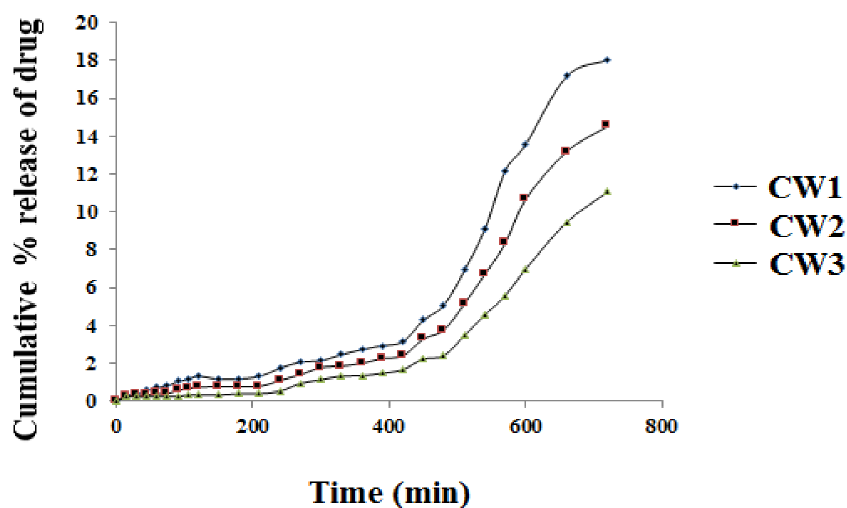

Fig-7.3. : Release of metronidazole from compression-coated tablets containing 10% w/w osmogen in core and LBG:CMLBG=50:50 in coat in different amounts

Table 7.14. : Cumulative % drug release from metronidazole compression-coated tablets containing 10% w/w osmogen in core and LBG:CMLBG=30:70 in coat in different amounts :

Time (min)	250 mg (CW1)		300 mg (CW2)		350 mg (CW3)	
	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
15	0.31±0.13	2.33±1.01	0.23±0.00	1.75±0.00	0.23±0.00	1.75±0.00
30	0.47±0.00	5.85±1.02	0.31±0.13	4.10±1.01	0.24±0.00	3.52±0.00
45	0.63±0.13	8.27±0.98	0.47±0.00	5.90±1.02	0.24±0.00	3.57±0.00
60	0.87±0.13	11.29±1.00	0.56±0.13	7.73±0.99	0.24±0.00	3.62±0.00
75	0.96±0.00	13.78±1.02	0.57±0.14	8.42±2.02	0.32±0.13	4.25±1.01
90	1.13±0.13	15.72±0.99	0.73±0.00	9.71±1.04	0.41±0.14	5.47±1.76
105	1.30±0.14	18.26±1.76	0.82±0.13	11.59±0.97	0.57±0.14	7.29±1.78
120	1.63±0.14	22.01±1.78	0.98±0.23	13.49±2.63	0.73±0.01	9.72±1.07
150	1.54±0.18	47.54±4.61	1.08±0.10	30.97±3.84	0.64±0.10	20.54±1.53
180	1.79±0.11	49.97±3.25	1.15±0.18	33.54±4.14	0.77±0.18	21.09±4.06
210	1.87±0.11	54.98±2.89	1.46±0.11	39.27±4.19	0.95±0.18	25.80±5.35
240	2.19±0.11	60.94±2.92	1.54±0.18	45.07±4.25	1.14±0.18	31.46±5.41
270	2.75±0.10	74.14±1.56	2.04±0.21	53.63±5.76	1.28±0.10	36.28±4.17
300	3.56±0.10	94.67±2.44	2.42±0.21	66.76±6.40	1.41±0.10	40.25±2.66
330	4.02±0.18	113.63±3.12	2.68±0.35	76.44±6.22	1.90±0.18	49.65±3.19
360	4.36±0.21	125.59±4.34	3.19±0.28	88.02±8.68	2.16±0.10	60.95±1.74
390	4.82±0.18	137.68±5.78	3.52±0.12	100.61±5.67	2.36±0.10	67.88±3.00
420	5.83±0.11	159.76±4.33	3.86±0.19	110.64±4.46	2.81±0.21	77.57±2.87
450	7.33±0.14	197.35±3.38	5.32±0.36	137.61±7.62	3.87±0.35	100.16±8.36
480	9.65±0.14	254.64±4.20	6.20±0.24	172.73±8.94	4.13±0.27	120.07±8.75
510	10.65±0.52	299.98±8.99	7.53±0.49	205.98±10.30	4.55±0.24	130.31±7.35
540	11.66±1.43	330.07±19.78	9.26±0.23	251.92±7.69	5.72±0.47	154.13±10.56
570	16.12±0.39	416.59±24.15	12.20±0.89	321.93±11.11	6.38±0.49	181.56±14.18
600	18.98±1.69	526.50±20.98	17.79±0.82	449.95±21.41	12.88±0.82	288.99±5.17

660	27.34±0.57	1389.67±61.42	24.05±1.36	1255.26±59.96	16.17±0.33	871.55±33.25
720	69.17±0.80	2895.38±41.12	35.31±0.78	1780.63±30.37	19.48±1.10	1069.50±41.99

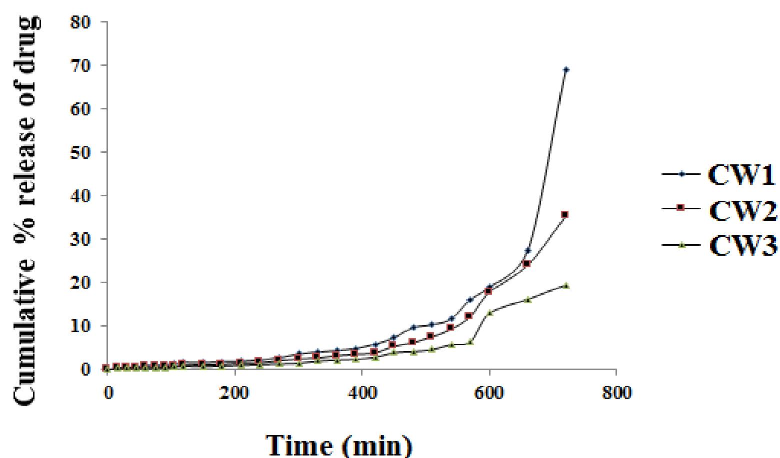

Fig-7.4. : Release of metronidazole from compression-coated tablets containing 10% w/w osmogen in core and LBG:CMLBG=30:70 in coat in different amounts

Table 7.15. : Cumulative % drug release from metronidazole compression-coated tablets containing 10% w/w osmogen in core and LBG:CMLBG=10:90 in coat in different amounts :

Time (min)	250 mg (CW1)		300 mg (CW2)		350 mg (CW3)	
	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)	Avg % drug release±S.D (n=3)	AUC mean± S.D (n=3)
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
15	0.54±0.13	4.08±1.01	0.23±0.00	1.75±0.00	0.23±0.00	1.75±0.00
30	0.78±0.14	9.96±2.03	0.39±0.13	4.68±1.01	0.24±0.00	3.52±0.00
45	0.80±0.14	11.84±2.06	0.79±0.14	8.82±1.76	0.24±0.00	3.57±0.00
60	1.12±0.14	14.34±1.81	0.87±0.13	12.44±1.76	0.40±0.13	4.78±1.01
75	1.13±0.14	16.87±2.08	0.89±0.14	13.20±2.03	0.48±0.00	6.60±1.02
90	1.38±0.14	18.85±2.10	1.05±0.14	14.55±1.81	0.88±0.14	10.18±1.04
105	1.71±0.24	23.18±2.80	1.22±0.24	17.07±2.74	1.04±0.14	14.40±1.78

120	2.04±0.37	28.15±4.57	1.39±0.14	19.64±2.75	1.21±0.01	16.92±1.05
150	1.93±0.20	59.56±8.47	1.35±0.19	41.20±4.80	0.96±0.01	32.35±0.15
180	2.31±0.20	63.51±5.97	1.37±0.19	40.76±5.72	1.09±0.11	30.85±1.70
210	2.63±0.30	74.04±7.41	1.80±0.29	47.47±7.18	1.34±0.18	36.56±3.15
240	2.96±0.23	83.80±7.80	2.12±0.20	58.74±7.26	1.60±0.11	44.13±4.13
270	3.17±0.11	91.85±4.67	2.44±0.29	68.33±7.30	1.85±0.10	51.77±0.19
300	3.62±0.82	101.78±2.43	2.76±0.09	78.02±3.31	1.99±0.11	57.70±2.68
330	4.13±0.44	116.30±5.35	3.15±0.29	88.71±4.48	2.25±0.11	63.69±2.86
360	4.42±0.30	128.28±10.53	3.30±0.20	96.81±6.88	2.64±0.28	73.33±5.82
390	5.12±0.24	143.07±8.04	3.70±0.20	104.99±6.11	2.90±0.17	83.07±3.29
420	6.19±0.34	169.68±8.70	4.27±0.21	119.54±6.17	3.05±0.20	89.32±5.34
450	8.37±0.24	218.36±6.85	6.05±0.37	154.90±8.02	4.39±0.34	111.57±7.91
480	9.88±0.26	273.62±7.31	6.64±0.37	190.42±9.85	4.88±0.13	139.00±6.86
510	10.80±0.26	310.16±5.99	8.21±0.50	222.71±13.06	5.75±0.23	159.53±2.01
540	12.49±0.27	349.32±7.96	9.71±0.55	268.82±15.64	6.11±0.13	178.03±2.04
570	17.41±0.51	448.38±11.56	13.63±0.61	350.22±17.09	8.12±0.60	213.56±7.12
600	21.63±0.39	585.56±13.23	19.09±0.39	490.88±14.92	12.92±0.82	315.70±20.64
660	29.94±0.67	1547.22±12.01	26.18±0.50	1358.18±26.86	19.65±2.00	977.14±60.55
720	89.17±1.09	3573.31±52.27	42.25±1.37	2053.03±55.74	26.82±0.67	1394.00±45.74

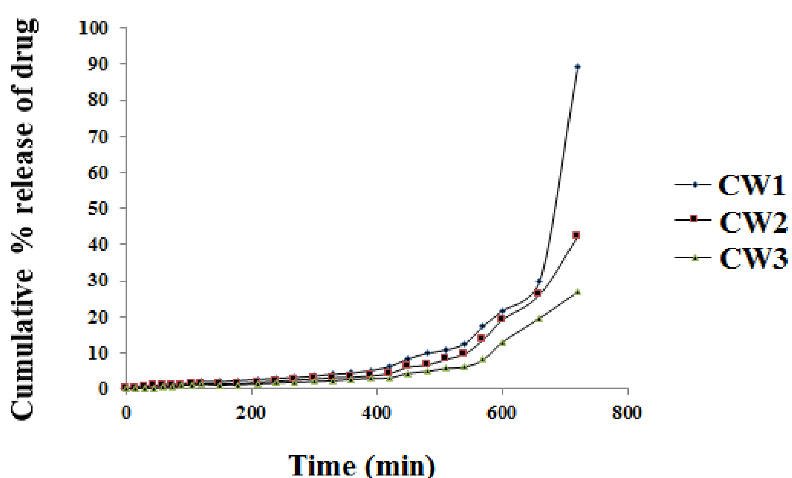

Fig-7.5. : Release of metronidazole from compression-coated tablets containing 10% w/w osmogen in core and LBG:CMLBG=10:90 in coat in different amount

PART VIII
DISCUSSION

8.1. Degree of substitution in carboxymethyl locust bean gum :

LBG is a non gelling neutral polysachharide which is highly viscous and relatively stable against variations in pH 3 to 11, salinity and temperature. Dissolved LBG adopts a disordered, fluctuating, random coil conformation. Galactomannans exist as poorly soluble "random coils", which need high temperature and vigorous agitation for complete dissolution in water. Carboxylated, hydroxylated and phosphate derivatives of galactomannans have been prepared to achieve better water binding capacity and to increase the water solubility.

Advantages of locust bean gum are (52) :

- It is biocompatible, biosorbable and biodegradable in nature.
- It is non-teratogenic and non-mutagenic according to Joint FAO/WHO Expert Committee on Food Additives held in Geneva, April '75
- It is cheap and abundant
- It has acceptable shelf-life
- Its degradation products are excreted readily
- It contains a number of derivatizable groups so that it can be modified to a derivative with better solubility and viscosity profile.

However, locust bean gum has certain limitations such as:

- Uncontrolled rate of hydration
- Microbial contamination
- Drop in viscosity on storage

Natural polymers, especially the polysaccharides contain numerous functional groups. These groups can be chemically modified. Modification of various functional groups impart newer properties to the parent gum and thus these modified polysaccharides find many diverse applications in drug delivery.

In general, negative charge induced transformation of natural polymer can be obtained by carboxymethylation, sulfonation and cyanoethylation. From the early stage of polymer science, the carboxymethylation has been accepted as the most easiest and useful method for introduction of carboxyl group into natural polymer containing polyhydric alcohol groups. In the present investigation, carboxyl groups were introduced in locust bean gum by a two step base catalyzed reaction procedure.

Degree of substitution (DS) is defined as the number of functional groups per unit residue of polymer chain that can be substituted by chemical modification. Because three reactive hydroxyl groups are present on each D-Glucopyranosyl unit, it is possible to introduce three sodium carboxymethyl groups per unit. Such a product would be described as having a degree of substitution of 3.00. When locust bean gum was modified to sodium carboxymethyl locust bean gum, the average degree of O-Carboxymethyl substitution was found to be 0.67 as shown in Table-7.2.

Probably, the 6-O is the most reactive site for carboxymethyl substitution. However, it is difficult to prepare carboxymethyl locust bean gum with a DS value higher than 1 by changing the NaOH concentration. A 50% NaOH solution seems to provide the optimum alkali concentration in the carboxymethylation process. At lower NaOH concentration, penetration of $ClCH_2COOH$ into the interlocking polymer is reduced. High alkali concentration (>60%) promoted side reaction between NaOH and $ClCH_2COOH$ and thus, the $ClCH_2COOH$ concentration decreased accordingly. Probably due to these reasons, the degree of o-carboxymethyl substitution was less than expected. The higher value of degree of substitution indicates more incorporation of carboxymethyl group.

8.2. Characterization of carboxymethyl locust bean gum :

FT-IR study of locust bean gum (Fig-7.1a) showed characteristic peaks at 3434.72, 2931.80, 1026.13, 873.75, 812.03 cm^{-1} indicating -OH stretching vibration formed by hydroxyl group of polysaccharide, C-H stretching vibration, C-O-C stretching vibration, β-linked-D-mannopyrannose units, α-linked-D-galactopyrannose unit, respectively. Several researchers have also reported the FT-IR spectra of LBG (51). FT-IR spectrum of carboxymethyl locust bean gum (Fig-7.1b) showed characteristic peaks at 3435.22, 2943.37, 1649.29 and 1408.04, 1327.03, 813.96, 875.68 cm^{-1}. This indicates -OH stretching vibration, C-H stretching vibration, C=O stretching in COO^- ions (asymmetrical and symmetrical vibration respectively), carbonyl stretching (C=O) of carboxymethyl moiety, α-linked-D-galactopyrannose unit, β-linked-D-mannopyrannose respectively. Also the peak at 1031.92 cm^{-1} in carboxymethyl locust bean gum was assigned to C-O stretching in C-O-C linkage. The presence of characteristic peak at 1327.03 cm^{-1} indicates carbonyl stretching (C=O) of carboxymethyl moiety and at 1649.14 and 1408 cm^{-1} for C=O stretching in COO^- ions (asymmetrical and symmetrical vibration respectively). This indicates the carboxymethylation

of locust bean gum. Similar FT-IR spectra of carboxymethyl locust bean gum was obtained during formulation on gastrointestinal delivery of glipizide from carboxymethyl locust bean gum–Al^{3+}–alginate hydrogel network (52). Similar FT-IR spectra of LBG was found during formulation of interpenetrating polymer network (IPN) microspheres for controlled drug delivery (53). Approximately the same FT-IR spectra of LBG was obtained during work on effect of hydrophilic natural gums in formulation of oral controlled release matrix tablets of propranolol HCl (54).

8.3. <u>Compatibility of drug with polymer</u> :

Interaction of drug with polymer in the compression-coated tablets was studied through FT-IR analysis. The spectra of pure MNZ and drug loaded compression-coated tablets with LBG and CMLBG blend in coatare shown in Fig-7.2. FT-IR spectra of MNZ showed the characteristics bands of -OH stretching, C-H &C=CH stretching, N-O stretching, -NO_2 symmetrical stretching and -C-O stretching respectively at 3223.05, 3101.54, 1537.27, 1369.46 and 1074.35 cm^{-1}. The spectra obtained from the powdered tablet showed the presence of the all above characteristic bands of the drug almost at the same wave numbers. These results indicate that apparently no drug excipient interaction occurred in the compression-coated tablets.

8.4. <u>Physical characterization of tablets</u> :

The compression-coated tablets of MNZ were prepared by wet granulation method using LBG and CMLBG blend in coat in order to have pulsatile release of the drug.

8.4.1. <u>Hardness of tablets</u> :

The results of tablet hardness have been shown in the Table-7.3. and Table-7.4. Tablet hardness or sometimes referred to as tablet crushing strength, is a very useful method for controlling bulk tablet production operation. The test is also called breaking-force testing which is the force required to break or damage the tablets in a specific plane. The suitability of a tablet with regard to mechanical stability during packaging and shipment can usually be predicted on the basis of hardness. The hardness of the core tablets was around 2 kg whereas hardness of compression-coated tablets was 4.0 to 6.5 kg.

8.4.2. Weight variation of tablets :

The tablets meet the weight variation test if the weight of not more than two tablets deviate from average weight more than the percentage limit given below and if no tablet differs by more than two times the percentage limit given below.

Table 8.1. : Percentage limit for weight variation of tablets :

Average weight of tablets	Percentage variation
130 mg or less	10
>130 mg but <324 mg	7.5
324 mg or more	5

The result was shown in Table-7.5 and Table-7.6. The weight variation of the tablets of each formulation was found to be within the permissible limits. Moreover, the values of standard deviation in the weight of the tablets in all the formulations were less. These results indicate minimum weight variation of the tablets in different formulation as well as within formulations.

8.4.3. Thickness of tablets :

Crown thickness uniformity is necessary not only from the consumers point of view but also for the packaging purpose. A ±5% deviation of the average thickness is allowed. The results of thickness measurement of different formulation were shown in Table-7.7 and Table-7.8. The tablets seem to have passed the test as the results indicated that variation in thickness was very less and well within ±5%.

8.4.4. Friability test of tablets :

Tablet friability results in weight loss of tablet in the packaging container owing to partial powdering, chipping or fragmentation of the tablet on attrition or wear. Tablets that are chipped or mechanically eroded have no longer sharp edges and lack the pharmaceutical elegance and havereduced quality. Tablet friability often reflects reduced cohesiveness on compression. It is one of the important factors which need much consideration for better designing of dosage forms. The results of tablet friability are shown in the Table-7.9 and

Table-7.10. The results are conforming to the compendia requirements as the % friability of all the tablets was well below 1% limit.

8.4.5. Content uniformity of tablets :

The weight variation test is not sufficient to assure uniform potency of tablets with low dose drugs. The greatest potential for non-uniformity of drug content in tablets is the segregation or drug-excipient separation which occurs in powders mixtures intended for direct compression and with wet granulation in which drug migration is very common. The results of content uniformity have been shown in the Table-7.11. The variation in drug content from the labeled potency was well within the ±15% limit permitted by different official compendia. In addition the standard deviation was also less. The results indicate that there was no segregation or drug-excipient separation during the tablet formulation stages.

8.4.6. Disintegration of tablets :

The disintegration time is the time necessary to disintegrate the tablet into small particles under standard conditions. However, it is not a bioavailability indicator. The results of disintegration tests are shown in Table 7.12. The results indicated that the core tablets disintegrated within 2 minutes and thus were considered as immediate release tablets.

8.5. In-vitro drug release studies :

The results of drug release from the compression-coated tablets having 10% osmogen in core tablets and blending with both native and modified gum in different ratios were shown in the Fig-7.3, Fig-7.4 and Fig-7.5.The different drug release data are based on the three different blending of LBG and CMLBG used in tablet coating and also on the three different coat weights of tablets as described in the Table-7.13, Table-7.14 and Table-7.15 respectively. The sigmoidal release pattern of MNZ showed initial lag period of around 7 hours and then a sudden burst release was found at 12 hours. Drug release study was carried out in continuous pH conditions at 37±0.5°C and 75 r.p.m. At first the tablets were immersed in 700 ml pH 1.2 acid solution mimicking stomach acidic environment and drug release studies were continued for 2 hours. Then the pH of the dissolution media was adjusted to pH 7.4 mimicking intestinal pH and the release studies were further continued upto 5 hours. Finally the pH was adjusted to 6.8 mimicking colonic pH and release studies were continued for upto 12 hours. When osmogen was fixed at 10% concentration in the core, the release of MNZ from the

compression-coated tablet followed the order LBG:CMLBG=10:90> LBG:CMLBG=30:70> LBG:CMLBG=50:50.

In case of LBG:CMLBG=50:50, drug release decreased with increasing coat weight. At 2 hours, tablets compression-coated with 250 mg, 300 mg and 350 mg polymer blend showed 1.31, 0.74 and 0.34% of drug release respectively. After 7 hours the release from the same was 3.15, 2.40 and 1.67% respectively. At the end of 12 hours the formulations showed 18.03, 14.53 and 11.06% drug release respectively.

In case of LBG:CMLBG=30:70, drug release decreased with increasing coat weight. At 2 hours, tablets compression-coated with 250 mg, 300 mg and 350 mg polymer blend showed 1.63, 0.98 and 0.73% of drug release respectively. After 7 hours the release from the same was 5.83, 3.86 and 2.81% respectively. At the end of 12 hours the formulations showed 69.17, 35.31 and 19.48% drug release respectively.

In case of LBG:CMLBG=10:90, drug release decreased with increasing coat weight. At 2 hours, tablets compression-coated with 250 mg, 300 mg and 350 mg polymer blend showed 2.04, 1.39 and 1.21% of drug release respectively. After 7 hours the release from the same was 6.19, 4.27 and 3.05% respectively. At the end of 12 hours the formulations showed 89.17, 42.25 and 26.82% drug release respectively.

However, in each coat weight, the release of MNZ was higher as the proportion of CMLBG in the blend was increased.

The pH and time dependent increase in drug release from the compression-coated tablets were also predicted by studying the area under the curves of cumulative % drug release vs. time profiles. The area under the curves was determined by trapezoidal rule. Higher value of area under thecurves indicates the more drug release from the compression-coated tablets. It was seen that the area under the curve for release of drug from compression-coated tablet of LBG:CMLBG=10:90 with 250 mg coat weight was higher than the area under the curves of all other formulations. (Table-7.14, Table-7.15, Table-7.16).

PART IX
SUMMARY & CONCLUSION

Metronidazole, a nitroimidazole,is the drug of choice for the treatment of infections caused by anaerobic bacteria and protozoa. Amoebiasis, is an infection of the lower G.I.T. caused by *Entamoeba histolytica*, a single celled protozoan parasite. The trophozoites of *Entamoeba histolytica* can invade the colonic epithelium causing amoebic colitis. Not only for amoebiasis, MNZ is also preferred in treatment of the lower G.I.T. diseases like, giradiasis, trichomonasis and anaerobic infections. This present work was aimed to develop a colon targeted compression-coated tablet which can minimize the drug release in gastric fluid and can release maximum amount of drug in the colonic fluid, rapidly and completely.

In the present study locust bean gum and its partially modified derivative sodium carboxymethyl locust bean gum were selected to formulate compression-coated tablets by wet granulation method. The resulting tablets were evaluated to assess whether such tablets are suitable for colon targeting. Locust bean gum was modified to sodium carboxymethyl locust bean gum by a two step base catalyzed reaction procedure. CMLBG was characterized by determining the degree of substitution and by FT-IR analysis. The degree of substitution was found to be 0.67. FT-IR spectra revealed the emergence of a new peak at 1327.03 cm^{-1} indicating caboxymethylation. The compatibility of the drug in the compression-coated tablets was evaluated by FT-IR analysis. The characteristic peaks of the drug were present in the spectra of compression-coated tablets with LBG and CMLBG blending in coat. This indicated that there was no interaction between the drug and the excipients of the tablets. The tablets were evaluated for hardness, weight variation, thickness, friability, content uniformity, disintegration and dissolution study. The tablets were found to comply with the pharmacopoeial requirements. Drug release study was carried out in continuous pH shift condition. The release of MNZ from the compression-coated tablets followed the order LBG:CMLBG=10:90> LBG:CMLBG=30:70> LBG:CMLBG=50:50 and 250 mg> 300 mg> 350 mg. The sigmoidal release pattern of MNZ showed initial lag period of around 7 hours and then a sudden burst release was found at 12 hours. It was found that the best formulation was compression-coated tablet of MNZ with 10% osmogen in core and LBG:CMLBG in 10:90 ratio with 250 mg coat weight as the premature drug release in upper G.I.T. upto 7 hours was less than 10% (6.19%) and maximum amount (89.17%) of drug was released subsequently in 12 hours. Disentangled structure of CMLBG led to higher swelling, producing higher release of drug. It is therefore concluded that a blend of LBG and CMLBG in appropriate amount may be used to prepare compression-coated tablets of MNZ and may be a useful strategy for pH and time dependent colonic delivery of the drug.

PART X
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