1.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is an idiopathic inflammatory disorder involving the mucosal and sub-mucosal part of the colon. Majority of IBDs which occur in humans are Crohn’s disease and ulcerative colitis (Hartmann et al., 2000). These two forms of the diseases are clinically related and they have histologically distinct chronic inflammation in the bowel, characterized by intermittent courses of acute attacks (Klein et al., 2005). In the last two decades there is an increase prevalence of ulcerative colitis and Crohn’s disease in Asian countries. (Dhar et al., 2011). In India the prevalence rate for ulcerative colitis was reported to be 44.3 / 100,000 individuals (Ahuja et al., 2010). While the age-standardized prevalence rates of colorectal cancer in India has been reported to be 3.2 and 4.2 per 100,000 for females and males respectively (Javid et al., 2011). The pathogenesis of IBD is incompletely understood. Genetic and environmental factors such as altered luminal bacteria and enhanced intestinal permeability play a role in the dysregulation of intestinal immunity, leading to gastrointestinal injury.

In Ulcerative colitis, formation of ulcers takes place in inner lining of large intestine or mucosa of the colon and rectum which results in diarrhea, blood and pus. Inflammation is very rigorous in the sigmoid and rectum and reduces in colon. Crohn’s disease is also known as regional enteritis, which is chronic inflammation of the intestine confined to the terminal portion of small intestine i.e. ileum. These Inflammatory Bowel Diseases have been linked with an increased risk of colorectal cancer. These diseases can be diagnosed by Blood test and Endoscopy. Increased number of white blood cells indicates the presence of inflammation (Mehta et al., 2011).

Considering the fact that, the etiology and inflammation inducing mechanisms in IBD are still poorly understood, the mainstay of medical treatment in IBD has remained the same four categories of drugs, namely sulfasalazine (1940s), steroids (1950s), immunosuppressives (1960s), Metronidazole and other antibiotics (1970s). However, development of new agents, better analogues of older agents, and improved modes of drug delivery offer great promise for the future of drug therapy of this disease (Talaei et al., 2013).

Environmental factors – Smoking, helminths, childhood infections, dietary habits, and psychosocial factors have all been implicated in the etiopathogenesis of IBD (Probert et al., 1992). Persons belonging to populations with a low incidence of IBD, on migration to developed countries, show a higher incidence of IBD, suggesting that environmental factors are important.
in IBD (Probert et al., 1991). The etiology of IBD is centered on the interaction of intestinal microflora with the host immune system (Tamboti et al., 2004).

Childhood infections induce immune tolerance to various extrinsic antigens by stimulating regulatory cells of the immune system, which have an anti-inflammatory activity (Bach, 2002). In epidemiological studies, reduction in the frequency of childhood infections has correlated with increase in autoimmune and allergic disorders (Wiss, 2002).

Following are some of the colonic diseases:

1.1.1. Colonic diseases

- Crohn’s Disease
- Ulcerative Colitis
- Diversional Colitis
- Ischemic Colitis
- Diverticular Inflammatory Bowel Disease
- Colon Cancer
- Lymphoma of Colon

Ulcerative colitis (UC) and Crohn’s disease (CD) affect mostly the colon tissue. Active inflammation causes the mucosa to become red and the mucous membrane appears erythematous, finely granular, and friable, with loss of the normal vascular pattern and often with scattered hemorrhagic areas. Colon inflammation mostly occurs in areas colored in red in both UC and CD (Fig. 1.1). While CD can be observed in different parts of intestinal tract, UC is observed in colon and usually rectum is also involved. A schematic view of inflamed colon tissue compared to normal colon tissue is illustrated in Fig. 1.1. Based on clinical/paraclinical parameters and symptoms encompassing abdominal spasms, rectal bleeding, anaemia, nausea, fever, fatigue, weight loss, appetite loss and tachycardia results, IBD is classified into mild, moderate and severe stages (Kawadkar and Ram, 2007). Although the association of IBD with mortality is not clearly known, this illness certainly affects the quality of life in patients. Hence, treatment of IBD based on maintenance of remission and prevention of recurrent relapse of inflammation has turned into a hot topic in recent decades (Kuhbacher and Folsch, 2007).
1.1.2. Anatomy and physiology of colon

GIT is divided into three parts i.e. stomach, small intestine and large intestine. Large intestine extends from ileocecal junction to the anus is divided into three main parts such as colon, rectum and anal canal. Colon is about 150 cm long and divided into five major segments (Mehta et al., 2011, Kothawade et al., 2011). Colon is cylindrical tube lined by a moist, soft pink lining called mucosa. Ascending and descending colon supports the peritoneal folds known as mesentery. The right colon consists of caecum, ascending colon, hepatic flexure and right half of transverse colon (Vandamme et al., 2002, Kopecek et al., 1992). The left colon contain the left half of the transverse colon, descending colon, splenic flexure and sigmoid. The rectum is last anatomic segment before the anus. The caecum and proximal colon act as fermentation chamber. The transverse colon holds material in the proximal colon and is important site for absorption of water. Proximal colon acts as a reservoir for faecal matter (Thomas et al., 1985, Basit, 2005).
The physiology of proximal and distal colon differs in many aspects related to their function and may affect drug absorption at each site (Table 1.1). The physical properties of the luminal contents of the colon also changes from liquid in the cecum to semisolid in the distal colon. There may also be differences in the environment of a drug whether it is in the bulk phase or next to the mucosa, and it is free in aqueous phase or trapped in solid material like dietary fiber residues (Sarasija and Hota, 2000, Bajpai et al., 2013).

Table 1.1: Differences between proximal and distal colon

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td>Fermentation chamber, absorption</td>
<td>Absorption, storage</td>
</tr>
<tr>
<td>Innervation</td>
<td>Vagal/pelvic Lumbar Muscle more distensible</td>
<td>Pelvic, lumbar, greater sensitivity to neural stimulation</td>
</tr>
<tr>
<td>Blood supply</td>
<td>Superior mesenteric Artery and vein, Greater blood flow</td>
<td>Inferior mesenteric Artery and Vein</td>
</tr>
<tr>
<td>Absorption</td>
<td>92% chloride dependent transport is electro neutral greater overall capacity</td>
<td>Chloride-dependent transport mainly, Amiloride sensitive</td>
</tr>
<tr>
<td>Luminal contents</td>
<td>Liquid, lower pH (4.6-7.8), Active microbial metabolism</td>
<td>Semisolid, neutral pH, lower bacterial activity</td>
</tr>
</tbody>
</table>

1.1.2.1. Major functions of colon:

- The indurations of intestinal contents into faeces by absorption of water and electrolytes and store the faeces until excretion. Absorption capacity of colon is very high. Everyday 2000 mL of fluid enters colon through ileocecal valve from which more than 90% of the fluid is absorbed.
- Colon has suitable environment for the growth of microflora like Bacteroids, Eubacterium and Enterobacteriaceae.
- Expulsion of contents of colon at suitable time.
- Colon absorbs water and Na\(^+\) from the lumen, concentrates the fecal content and secretion of K\(^+\) and HCO\(_3\) (Kothawade et al., 2011).
1.1.2.2. The pH of GIT

The pH in stomach, small intestine and colon is different due to the factors like diet, food intake, intestinal motility and disease states (Spitacl et al., 1980, Devereux et al., 1990). It is very difficult to design a delivery system which is robust enough to withstand changes of gastric pH (Thomas et al., 1985). The diagram human gastro-intestinal tract pH range chart illustrates the pH variation in gastro-intestinal duct along with the average time food spends in each section (Fig. 1.2 and Table 1.2). The lowering of pH has to be targeted to deliver the drug to small intestine by pH sensitive enteric coatings (Evans et al., 1988, Tomlin and Read, 1988).

![Human gastro-intestinal tract pH range chart along with transit time](Reprograph from Talaei et al., 2013)
### Table 1.2: pH of different parts of GIT

<table>
<thead>
<tr>
<th>Location</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>1.0-2.0</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>3.0-5.0</td>
</tr>
<tr>
<td>Jejunum</td>
<td>5.0-6.5</td>
</tr>
<tr>
<td>Ileum</td>
<td>6.0-7.5</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>6.4</td>
</tr>
<tr>
<td>Colon</td>
<td>6.7-7.3</td>
</tr>
</tbody>
</table>

#### 1.1.2.3. Microflora of colon and enzymes

A variety of aerobic and anaerobic bacteria are present in human GIT. Intestinal enzymes help in release of drug efficiently in various parts of GI tract. These enzymes are produced from the microflora present in colon (Basit, 2005). These enzymes enhance the drug release by breaking bonds between the inert carrier and active ingredient. About 400 bacterial species are present such as *Bacteroides, Bifidobacterium, Peptococcus, Ruminococcus* and *Clostridium*. Endogenous and exogenous substrates, such as carbohydrates and proteins, bypass digestion in the upper GIT and get metabolized by the enzymes secreted by colonic microflora (Table 1.3) (Molly et al., 1993).

### Table 1.3: Various enzymes and their metabolic reactions

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Enzymes</th>
<th>Metabolic reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli, Bacteroides</em></td>
<td>Nitroreductase</td>
<td>Nitro to amino or azo.</td>
</tr>
<tr>
<td><em>Clostridia, Lactobacilli, E. coli</em></td>
<td>Azoreductase</td>
<td>Reductive cleavage of azo compounds.</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>N-Oxide reductase, sulfoxide</td>
<td>Reduce N-oxides and sulfoxides</td>
</tr>
<tr>
<td><em>Clostridia, Lactobacilli</em></td>
<td>Hydrogenase</td>
<td>Reduce carbonyl and aliphatic double bonds</td>
</tr>
<tr>
<td><em>E. coli, P. vulgaris, B. subtilis, B. mycoides</em></td>
<td>Esterases and amidases</td>
<td>Cleaves esters</td>
</tr>
<tr>
<td><em>Eubacteria, Clostridia, Streptococci</em></td>
<td>Sulfatase</td>
<td>Cleaves O-sulfates and sulfamates</td>
</tr>
</tbody>
</table>
1.1.2.4. Absorption of drug in colon

Absorption of drug in the colon takes place by two routes such as Paracellular and Transcellular route. Mostly the absorption of lipophilic drugs occurs through the transcellular route. On the other hand absorption through paracellular route involves the transfer of hydrophilic drugs through tight junctions between the cells (Mahale et al., 2013, Basit, 2005). The oral absorption of peptide and protein drugs is limited because of degradation in acidic environment of stomach, enzymatic degradation in small and large intestine, rapid transit from small intestine, low mucosal permeability and extensive first pass metabolism by absorbing membrane and the liver (Challa et al., 2011, Mehta et al., 2011 and Basit, 2005).

1.1.2.5. Barriers in colon absorption

A number of barriers are responsible for limiting the absorption of drug to colon. In the lumen, when the specific and non-specific drug binding occurs due to the interaction of drug with the dietary components which cause a barrier in drug absorption. Non-selective interactions could occur between regions of glycoprotein drug and undigested food stuffs such as waxes and alginates. The mucus layer at the epithelial surface is highly charged and sieve like nature acts as a formidable thermodynamic barrier for transit of large and negatively charged drug molecules. These barriers enhance the colonic residence time and environmental or enzymatic degradation. Removal of mucus barrier using mucolytic agents seems attractive which may implicate in variety of disease processes and pathological conditions due to alteration of intact mucus layer (Bajpai et al. 2003).

1.1.3. General considerations for designing of colonic formulations

Colonic formulations should be designed in such a way that they provide a ‘burst release’ or sustained/prolonged release when they reach the colon. Designing a formulation depends upon various factors like pathology, pattern of disease and affected parts of the lower GIT or physiology and physiological composition of healthy colon if formulation is not intended for localized treatment, physiochemical and biopharmaceutical properties of the drug like solubility, stability and permeability at intended site of delivery and the desired release profile of active ingredient (Kothawade et al., 2011).
Presently, the inflammatory diseases like chronic colitis, ulcerative colitis and Crohn’s diseases are treated with glucocorticoids namely dexamethasone and methyl prednisolone and many anti-inflammatory drugs by oral and parenteral route produces systemic side-effects including adenosuppression, immunosuppressant, cushinoid symptoms and bone resorption. Therefore, if colon targeted drug delivery system is used, it decreases the therapeutic dose and reduces the systemic side-effects caused due to high doses (Kulkarni et al., 1999, McLeod et al., 1994).

Conventional therapies for colon diseases are not very effective, as the drug does not reach the site of action at therapeutic concentration. Therefore, this treatment requires relatively large doses to compensate drug loss during passing GIT which causes undue side-effects. Due to this, colon-specific drug delivery system is required (Paharia et al., 2007).

Colon specific drug delivery system is an oral-colonic delivery system which shows negligible release of drug in stomach and allows the complete and controlled release of drug in lower GI tract i.e. colon (Vajpayee et al., 2011, Challa et al., 2011). This delivery system is beneficial in case of drugs needed to be protected from hostile environment of upper GI tract. The colon is a site where both local and systemic delivery of drugs can be given. Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn’s disease and colonic cancer (Chourasia and Jain, 2003, Challa et al., 2011). However the drug can be targeted directly into the colon to reduce the systemic side effects, dose of administration, improves the efficacy and patient compliance (Chourasia and Jain, 2003). Colon specific drug delivery is used in chronotherapy, prophylaxis of colon cancer and in treatment of nicotine addiction also (Bajpai et al., 2003 and Challa et al., 2011). Precise colon targeted drug delivery requires that the triggering mechanism in the delivery system should only respond to the physiological conditions particular to the colon. Hence, continuous efforts should be focused on designing colon-specific delivery systems with improved site specificity and drug release kinetic to accommodate different therapeutic needs.

1.1.4. Pharmaceutical approaches for colon specific drug delivery system

1.1.4.1. Covalent linkage of drug with carrier

Prodrug approaches: Prodrug is a pharmacologically inactive derivative of a parent molecule that requires enzymatic transformation in the biological environment at the target site to release the active drug (Liu et al., 2011). For colon drug delivery, the prodrug is designed to undergo minimal hydrolysis in the upper part of GI tract and it undergoes enzymatic hydrolysis in the
colon thereby releasing the active drug moiety from the drug carrier. Prodrugs are new chemical entities and require various evaluations before being used as a carrier (Basit et al., 2004).

**Polymeric prodrugs:** For delivery of the drug to the colon, newer prodrugs are aimed to use the polymer as drug carrier (Kulkarni et al., 1999). Both synthetic and naturally occurring polymers have been used for the colon drug delivery system. Sub synthetic polymers can be used to form polymeric prodrug with azo linkage between the polymer and drug moiety. Coating the capsules with peptide polymers cross linked with azo aromatic group had been found to protect the drug from digestion in the stomach and small intestine (McLeod et al., 1994). The drug release takes place due to the reduction of azo bonds (Huttunen and Rautio, 2011).

**Hydrogels:** The synthesis and characterization of a series of novel azo hydrogels for colon targeted drug delivery have been described by Brondsted and Kopecek. With the presence of pH-sensitive monomers and azo-cross-linking agents in the hydrogel structure, the colon specificity can be achieved. During the transit through the GI tract, the swelling capacity of the hydrogel increases with increase in pH and being highest around pH 7.4. The hydrogel reaches to a degree of swelling that makes the cross-linking accessible to the enzymes or mediators upon arrival in the colon. The degradation of the cross-linking results in the release of drug from the disintegrated gels. By conducting *in-vitro* degradation studies in rat caecal content medium and *in-vivo* degradation studies by implanting in the stomach and caecum of male rats, the performance of the hydrogels for colon specific drug delivery can be evaluated.

**1.1.4.2. Pressure dependent drug delivery:** The digestive processes involve contractile activity of stomach and peristaltic movements for propulsion of intestinal contents in the gastrointestinal tract (Vyas and Khar, 2005). As a result of peristalsis, higher pressures are encountered in the colon than in the small intestine which forms the basis for design of pressure controlled system (Hita et al., 1997). Takaya *et.al.* had developed pressure controlled colon delivery capsules made up of ethyl cellulose, which was water insoluble. By changing the size of the capsule and thickness of the capsule shell wall, the system can be modified to withstand the rupture at different pressure. Lag time of three to five hours in relation to drug absorption should be noted when pressure controlled capsules will administered to humans (Antonin et al., 1996).

**1.1.4.3. Time dependent delivery:** For targeting of drug to colon, time dependent delivery has been proposed. Time dependent systems release their drug load after a preprogrammed time delay until the drug reaches the colon (Chickpetty et al., 2011). The lag time in this case is the
time required to transit the drug from mouth to colon (Muaola et al., 1998). Time controlled systems are useful for synchronous delivery of a drug either at a pre-selected time such that patient receives the drug when needed or at a pre-selected site of the GI tract. Design of enteric coated time dependent press coated tablet is shown in Fig. 1.3. The spatial targeting of the drug to colon generally follows pulsatile release. Pulsatile release profile is characterized by the initial lag time followed by rapid and complete drug release (Fukui et al., 2000).

![Fig. 1.3: Design of enteric coated timed-released press coated tablet](image)

1.1.4.4. **Pulsatile drug delivery system**: Pulsatile drug delivery system can be classified into site-specific and time controlled system. Drug release from site-specific system depends on the gastro intestinal environment (Kinger et al., 1998). Time controlled drug delivery systems are independent of the biological environment (Fig. 1.4).

![Fig. 1.4: Classification of pulsatile drug delivery system](image)
Single unit time controlled drug delivery system

i) Pulsincap was the first formulation based on this principle (MacNeil et al., 1990, Hebden et al., 1999). It is same as that of hard gelatin capsule in appearance. In one method the body of a hard gelatin capsule can be filled with organic acid as a pH- adjusting agent. Ethanolic solution of ethyl cellulose can be used to seal the joint of the capsule. First coating of the capsule can be done with an acid soluble polymer (Eudragit E) followed by a hydrophilic polymer HPMC (hydroxyl propyl methyl cellulose) coating and finally enteric coated with Eudragit L (Watanabe et al., 1998). The outermost enteric layer of the coating will prevent drug release in the stomach after ingestion of the capsule. Coated layers (enteric and hydrophilic) dissolve quickly and water starts entering the capsule after reaching colon. By the dissolution of organic acid, when the environmental pH inside the capsule decreases, the acid soluble layer dissolves and the enclosed drug release rapidly. Therefore by modifying the thickness of acid soluble layer, the onset time of drug release in the intestine can be controlled.

ii) Time Clock, a delivery system has been exploited for drug release in the colon. It consists of solid dosage form coated with a hydrophobic surfactant layer and hydrophilic polymer is added to improve adhesion to the core (Steed et al., 1997). In the aqueous environment, the outer layer re-disperses in a time proportional to the thickness of the film and then the core is available for dispersion.

1.1.5. Multiple unit time controlled drug delivery system

In multiple unit system pellets can be prepared in conventional coating pan. Coating of drug pellets can be done with an inner layer of a combination of two pH independent polymers Eudragit RL and Eudragit RS and an outer layer of Eudragit FS, pH dependent polymer (Sareen et al., 2013).

1.1.5.1. pH - dependent systems

Enteric-coated or pH sensitive polymer coated drug delivery systems have been incorporated in this disease, where drug release is triggered by the sensitivity of polymer to luminal pH during GI transit. The coating polymers should be anionic, insoluble in acidic pH and be swellable or soluble in pH levels higher than 6.8 (Rodriguez et al., 1998). The most commonly used polymers
are Eudragit (polymethacrylate polymers) and cellulose Acetate Phthalate (CAP) which have been employed for colonic delivery against IBD. Optimum pH for dissolution of various enteric polymers is shown in Table 1.4.

**Table 1.4: Optimum pH for dissolution of various enteric polymers**

<table>
<thead>
<tr>
<th>Enteric Polymers</th>
<th>Optimum pH for dissolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvinyl acetate phthalate</td>
<td>5.0</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, type A</td>
<td>≥6.0</td>
</tr>
<tr>
<td>Eudragit</td>
<td>&gt;7.0</td>
</tr>
<tr>
<td>Hydroxypropylmethylecellulose phthalate</td>
<td>≥5.5</td>
</tr>
<tr>
<td>Cellulose acetate trimelitate</td>
<td>5.5</td>
</tr>
<tr>
<td>Hydroxypropylmethylecellulose acetate succinate</td>
<td>≥6.0</td>
</tr>
<tr>
<td>Shellac</td>
<td>7.0</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type B</td>
<td>≥7.0</td>
</tr>
</tbody>
</table>

1.1.6. **Bacteria dependent colon drug delivery:** Inside the human gastrointestinal tract the bioenvironment is characterized by the presence of complex micro flora especially the colon that is rich in microorganisms which are involved in the process of reduction of dietary component or other materials (Cole et al., 2002). The micro flora of colon is in the range of 10^{11}-10^{12} CFU/ml. A large number of aerobic and anaerobic bacteria are present in the entire length of the human GI tract. Over 400 distinct bacterial species have been found and out of which 20-30% are of genus bacteroids. The upper region of the GI tract has a very small number of bacteria and predominantly consists of gram positive facultative bacteria. The rate of microbial growth is greatest in the proximal area because of high concentration of energy source. Polymers can be used to coat the drugs, which show degradability due to the influence of colonic micro-organism, can be exploited in designing for colon targeted drug delivery (Cui et al., 1994).

1.1.7. **Osmotic controlled drug delivery system:** For the treatment of diseases of colon, OROS-CT can be used to target the drug locally to the colon. This system can be a single osmotic unit or 5-6 push-pull units encapsulated within a hard gelatin capsule. When this system enters into small intestine, at high pH (pH > 7), the coating dissolves and water enters inside the unit leading to the swelling of osmotic push compartment (Sareen et al., 2012). This swelling forces drug gel
out of the orifice at a controlled rate through the semi permeable membrane. Each unit can be
design with a 3-4 hour post gastric delay to prevent the delivery of drug in the small intestine for
treating the ulcerative colitis. Table 1.5 showed different factors to be considered in designing
the colon drug delivery system.

Table 1.5: Factors considered in the design of colon drug delivery system

<table>
<thead>
<tr>
<th>Formulation Design</th>
<th>Factors affecting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Coating with polymer</td>
<td></td>
</tr>
<tr>
<td>i. Coating with pH- polymer</td>
<td>Formulation coated with enteric polymers release drug when pH moves towards alkaline range</td>
</tr>
<tr>
<td>ii. Coating with biodegradable polymer sensitive</td>
<td>Drug is released following degradation of the polymer due to the action of colonic bacteria</td>
</tr>
<tr>
<td>2. Embedding in matrices</td>
<td></td>
</tr>
<tr>
<td>i. Embedding in biodegradable polysaccharides</td>
<td>The embedded drug in polysaccharide matrices is released by swelling and biodegradable action of polysaccharides.</td>
</tr>
<tr>
<td>ii. Embedding in pH sensitive matrices</td>
<td>Degradation of pH sensitive polymer in the GIT releases the embedded drug</td>
</tr>
<tr>
<td>3. Timed released systems</td>
<td>Delaying the release of the drug until it enter into the colon</td>
</tr>
<tr>
<td>4. Bioadhesive system</td>
<td>Drug coated with bioadhesive polymer that selectively provides adhesion to colonic mucosa</td>
</tr>
<tr>
<td>5. Coating of microparticles</td>
<td>Drug is released through semipermeable membrane</td>
</tr>
<tr>
<td>6. Osmotic controlled delivery</td>
<td>Osmotic pressure</td>
</tr>
</tbody>
</table>

1.1.8. Polysaccharide based delivery systems

Polysaccharides are present in abundance, are inexpensive, can be easily modified chemically,
biochemically and are stable, non-toxic, hydrophilic, safe, gel forming and biodegradable.
Naturally occurring polysaccharides are guar gum, inulin, chitosan, chondrotin sulphate,
alginites, and dextran. Colonic microflora can metabolize these polysaccharides to simple
saccharides. These polysaccharides come in the category of ‘generally regarded as safe’ (GRAS).
Chitosan is obtained from chitin in crab and shrimp shells by deacetylation which is degraded by
colonic microflora (Ashord et al., 1993). Inulin natural polysaccharide obtained from onion and
garlic undergoes colonic degradation. Polysaccharides which degrade in colonic environment are
shown in Table 1.6.
Table 1.6: Microbially degradable polysaccharides for colon targeting

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disaccharides</td>
<td>Lactose, Maltose</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Celllobios, Cyclodextrins, Lactulase, Raffinose, Stachyose</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Alginates, Amylose</td>
</tr>
</tbody>
</table>

1.1.9. CODES Technology (Li et al., 2002)

CODES technology was designed for colon-specific drug delivery. This technology has advantages of some polysaccharides that are metabolized by microflora present in colon which is coupled with pH sensitive polymer coating (Fig. 1.5). This delivery system avoids the problems associated with pH and time dependent delivery systems. This system has the ability to achieve colon delivery consistently and reliably as the degradation of polysaccharides mainly takes place in colon (Watanabe et al., 1998).

![Fig. 1.5: Schematic presentation of CODES formulation](image-url)
This system is developed by using lactulose which triggers the colon-specific delivery of drug. System consists of lactulose as core material in tablet, which is coated with acid soluble material, Eudragit E and subsequently over coated with an enteric material, Eudragit L. This enteric coating protects the tablet from environment of stomach and dissolves rapidly after gastric emptying. Then the acid soluble coating protects it from alkaline pH of small intestine and when tablet arrives in colon, the microflora enzymatically degrades the polysaccharide [lactulose] into organic acid. Due to which pH surrounding the system lowers and acid soluble coating solubilizes and increases the drug release (Surh et al., 2001, Yang et al., 2002).

1.1.10. Colonic drug delivery system based on pectin and galactomannan coating
This system consists of a conventional tablet or capsule coated with two specific polysaccharides like pectin and galactomannan. Individually they are not used as a drug carrier for colon-specific delivery due to their high water solubility/swelling characteristics. The coatings produced from the mixture of two polysaccharides show solubility depending upon the pH of the coating solution (Lee et al., 1999). The coating from a pH 7 aqueous solution of pectin and galactomannan was shown to be strong, elastic and insoluble in simulated gastric and intestinal fluids. Therefore, the coating of such polymer could protect drug from being released in the upper GIT. On the other hand, the coating from the identical solution with pH 7 observed to dissolve readily in the simulated intestinal fluids. Due to the hydrogen bonding, hydrophobic force and the formation of an inter-junction zone a complex between the two polysaccharides that might have been formed at pH 7 that lead to conformational changes in polysaccharides at the higher pH. Higher percentage of galactomannan results in decreased bacterial degradation in the colon and prolonged duration of action and also of negligible drug release in the upper GIT (Wakerly et al., 1996, Basit, 2000).

1.1.11. Multiparticulate system based drug delivery
Multiparticulate dosage forms have more potential benefits in comparison to single unit systems such as increased bioavailability, reduced systemic toxicity, reduced local irritation and predictable gastric emptying. Multiparticulate approaches tried for colonic delivery includes the formation of pellets, granules, micro-particles and nanoparticles. The use of multiparticulate drug delivery system in preferred to single unit dosage forms as it has been observed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the
ascending colon for a relatively long period of time (Kramer et al., 2003, Hardy et al., 1985). Main multiparticulate systems for colon specific delivery include pellets, granular matrices, beads, microspheres, microsponges and nanoparticles (Zambito et al., 2005, Rodriguez et al., 1998 and Davis, 1989).

Microspheres as colon specific drug delivery system has several advantages over the single unit delivery system, as microspheres can:

(i) provide more uniform drug dispersion in the gastrointestinal tract and more homogeneous drug absorption
(ii) lower inter- and intra-individual variability
(iii) prolong the colonic residence time
(iv) Reduces the local irritation.

Furthermore, as compared to conventional drug delivery system, microspheres-based colon specific drug delivery system provides more uniform and reproducible transit through gastrointestinal tract (Chandran et al., 2009).

1.1.12. New Theoretical Background

IBD treatment involved high doses of immunomodulator, steroids and surgery produces systemic side effects. Research on compounds for the prevention of IBD is going on and the most promising group of compounds for therapy include the nonsteroidal anti-inflammatory drugs (NSAIDs) as they are known to inhibit of the cyclooxygenase (COX) enzymes, which are involved in metabolism of arachidonic acid and the production of various eicosanoids but NSAIDs has reported to cause some upper GIT adverse effects which cannot be avoided.

Flurbiprofen (FLB) is an NSAID with well-known anti-inflammatory, antipyretic, and analgesic properties. FLB has a plasma half-life of 3–6 h. Due to short half-life frequent dosing is needed to maintain the therapeutic efficacy for an extended time. The primary goal of the drug therapy for IBD is to reduce inflammation in the colon, requiring frequent intake of high doses of NSAIDs, which may lead to gastric ulceration, bleeding, and other gastric complications.

In recent years the natural compound curcumin has gained prior attention for its multiple pharmacological effects. Curcumin is a major diphenolic pigment found in turmeric root \([Curcuma longa\ L., Family: Zingiberacea]\). Curcumin (natural drug) has been used extensively in Ayurvedic medicine for centuries, as it is nontoxic and has a variety of therapeutic properties.
including anti-oxidant, analgesic, anti-inflammatory and antiseptic activity. It has also been reported to show antibacterial, wound healing, hypocholesterolemic, anticoagulant, antispasmodic and hepatoprotective activities. More recently curcumin has been found to possess anti-cancer activities via its effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis. Various clinical studies have suggested that curcumin might be a potential candidate for the prevention and/or treatment of a variety of colonic diseases such as ulcerative colitis, Crohn’s disease and colonic cancer.

Curcumin may confer some additional therapeutic advantages when used in combination with certain conventional anti-inflammatory medicines. Curcumin possesses anti-inflammatory activity, which involves inactivation of both the lipoxygenase and cyclooxygenase enzyme systems and hence can be useful in suppressing carcinogenesis, since any drug that can inhibit both the lipoxygenase and cyclooxygenase pathways would also suppress leukotrienes (Fig. 1.6).

COX: Cyclooxygenase; HPETE: Hydroperoxyeicosatetraenoate; LOX: Lipoxygenase; LT: Leukotriene; PL: Phospholipase; PG: Prostaglandin; TX: thromboxane.

Fig. 1.6: Anti-Inflammatory Effect of Curcumin by Inhibition of Arachidonic Acid Pathway
1.2. Objectives of the Research

Inflammatory bowel diseases (Crohn’s disease and ulcerative colitis) are non-specific inflammatory conditions of alimentary tract. The former can affect any part of the alimentary tract whereas ulcerative colitis is confined to the large intestine. The diagnosis of IBD requires a comprehensive physical examination and a review of the patient’s history. Various tests, including blood tests, stool examination, endoscopy, biopsies, and imaging studies help in the diagnosis of disease.

Based on clinical/paraclinical parameters and symptoms encompassing abdominal spasms, rectal bleeding, anemia, nausea, fever, fatigue, weight loss, appetite loss and tachycardia results, IBD is classified into mild, moderate and severe stages (Kawadkar and Ram, 2007). Hence, treatment of IBD based on maintenance of remission and prevention of recurrent relapse of inflammation has turned into a hot topic in recent decades (Kuhbacher and Folsch, 2007). Further, in therapeutic regimens utilizing oral administration, systemic absorption in upper GIT is an undesirable delivery feature for IBD drugs, hence, dosage forms which are targeted to diseased bowel tissue could provide direct exposure of a therapeutic agent to sites of pathology, reduce its side effects and finally improve its therapeutic effect. The application of common therapeutic approaches in IBD therapies depends primarily on the severity of the disease and the area involved.

IBD management often requires long-term treatment based on a combination of drugs to control the disease. The common therapeutic agents normally include but are not limited to: aminosalicylates (first line treatment), corticosteroids (second line treatment) and immunosuppressives (third line treatment).

Albeit the effectiveness of these agents in management of inflammation, the harmful side effects observed through all the above treatments cannot be neglected (Kuhbacher and Folsch, 2007). So to overcome this nutraceuticals which are known to show their marked effect on colitis can be utilized. Curcumin is the most suitable candidate among them. Curcumin is a natural polyphenolic compound present in turmeric exhibited multiple pharmacological activities. Extensive studies in last two decade suggested that curcumin possesses anti-inflammatory, anticancer, antiviral, anti-amyloid, antiarthritic and antioxidant properties. The mechanism for these effects involves modulation of several signaling transduction pathways. Various clinical studies have suggested that curcumin might be a potential candidate for the prevention and/or treatment of a variety of colonic diseases such as ulcerative colitis, Crohn’s disease and colonic
cancer. However, several evidences suggested the role of curcumin in multiple diseases, but the major challenge is to obtain optimum therapeutic levels of curcumin due to its low solubility and poor bioavailability. Complexation of curcumin with transition metals is one of the useful requirements to overcome the problem related with solubility, bioavailability and stability. Curcumin can chelate various metal ions to form metallocomplexes of curcumin, which show greater effects than curcumin alone. Curcumin may confer some additional therapeutic advantages when used in combination with certain conventional anti-inflammatory medicines. Flurbiprofen is an NSAID with well-known anti-inflammatory, antipyretic, and analgesic properties. It is a propionic acid derivative. Its short biological half-life of 3-4 h (Kean et al., 1992, Davies, 1995) means frequent dosing is needed to maintain the therapeutic efficacy for an extended time. The main goal of the drug therapy for IBD is to reduce inflammation in the colon, requiring frequent intake of high doses of NSAIDs, which may lead to gastric ulceration, bleeding, and other gastric complications (Dhaneshwar et al., 2007). So it is hypothesized that a combination therapy containing curcumin and flurbiprofen will show synergistic action with lesser doses.

Drug delivery to colon encounters obstacle such as absorption and degradation in the upper GIT (Mrsny, 1992). The optimum drug delivery to colon requires avoidance of the absorption of these drugs from the small intestine. Over the last decade various different approaches have been studied to achieve drug targeting to colon viz., time dependent, pH dependent, microflora or enzyme activated system and pressure controlled based systems (Patel et al., 2007). An optimum colon targeted drug delivery ensures direct treatment at the disease site and thus lowers the dose and reduces systemic side effects. Multiparticulate system was selected since it offers slower passage rate through the colon and its higher surface area is advantageous for local treatment in colon. Moreover microspheres-based colon specific drug delivery system provides more uniform and reproducible transit through gastrointestinal tract (Chandran et al., 2009). Microspheres as colon specific drug delivery system has several advantages over the single unit delivery system, as microspheres can: (i) provide more uniform drug dispersion in the gastrointestinal tract and more homogeneous drug absorption, (ii) lower inter- and intra-individual variability, (iii) prolong the colonic residence time, and (iv) reduces local irritation (Jose et al., 2011).
1.2.1. Aims & Objectives

The aim of the work is to utilize colon targeted drug delivery systems for the treatment of inflammatory bowel disease for better therapeutic effects, reduction in dose and side effects. Thus, our specific objectives are:

- To bring our new therapeutic mode for IBD.
- To carry out preformulation studies of Curcumin and Flurbiprofen in order to evaluate its identity, compatibility with excipients and solubility.
- To formulate and evaluate multiparticulate system (microspheres) containing Curcumin.
- To prepare and characterize Curcumin-Zn(II) complex for increasing its solubility and stability.
- To formulate and evaluate multiparticulate system (microspheres) containing Curcumin-Zn(II) complex.
- To formulate and evaluate multiparticulate system (microspheres) containing Flurbiprofen.
- Pharmacodynamic analysis of developed formulations.
  - Experimental ulcerative colitis using acetic acid induced model.
  - Organ bio-distribution study.
  - Histopathological studies.
- Pharmacodynamic analysis of combination of Curcumin-Zn(II) complex microspheres and Flurbiprofen microspheres.
- Acute oral toxicity study of combination of Curcumin-Zn(II) complex microspheres and Flurbiprofen microspheres.
- Pharmacokinetic analysis of developed formulations.
- In vitro- In vivo correlation of developed formulations.

This study would thus:

Advance the knowledge of the effect of curcumin, its complex and flurbiprofen in treating IBD. It would pave the way for better treatment by site specific delivery of curcumin, its complex and flurbiprofen to colon.