CHAPTER 1

"INTRODUCTION"

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1.1 INFLAMMATORY BOWEL DISEASES (IBD)

These gastrointestinal inflammatory disorders are idiopathic, chronic, and recurrent, including ulcerative colitis and Crohn's disease (Messaris *et al.*, 2019). The small and large intestines and the colon are the main organs involved in inflammatory bowel illnesses, characterized by persistent inflammation in a specific mucosal or transmural region (Chey *et al.*, 2015). Proper local targeting is a significant difficulty in treating ulcerative colitis (UC). A well-designed drug delivery system is advantageous for better localization and therapeutic efficacy. The disease's focus on the drug's delivery system is a crucial threat to developing therapeutically efficient strategies for IBD (Brusini *et al.*, 2020).

For local therapy of inflammatory bowel illnesses such as UC, Crohn's disease, amebiosis, and colon cancer, targeted drug delivery into the colon is hugely desirable. Oral controlled release drug delivery is a system that allows for the continuous oral delivery of drugs with predictable and uniform kinetics for a predetermined time during GI transit, as well as the delivery of a drug to a specific area within the digestive tract for either local or systemic activity (Chen *et al.*, 2021). For the local treatment of various intestinal illnesses, the oral route is the most valuable and appropriate (Baumgart *et al.*, 2009). Rectal administration is the most precise way to deliver medications to the colon. Rectal administration is difficult to reach the proximal region of the colon. Rectal administration can sometimes be inconvenient for patients, resulting in lower-than-optimal compliance (Philip *et al.*, 2010).

Currently, the cause of UC is unknown; however, it is thought that genetic, gut/environmental, psychosomatic, autoimmune, and epidemiological variables are all implicated in the disease's progression (Pokorny *et al.*, 2001).

Immune/epithelial interactions, bacterial infections, and epithelial blockade activities

are all influenced by gut/environmental variables (Yadav *et al.*, 2009). Dietary habits, smoking habits, drug intake, hormonal conditions, climatic variation, and social context differences are all part of an epidemiological study (Ananthakrishnan *et al.*, 2013).

Several cell signaling pathways, inflammatory mediators such as tumor necrosis factor (TNF-), Interleukin-1, Interleukin-6, Interleukin-12, Interleukin-4, Interleukin-10, and Interleukin-11, Eicosanoids profiles, and others can be used to assess inflammatory features (Torres *et al.*, 2012). Chronic inflammation caused by reactive oxygen species causes dysplasia, or UC-associated colorectal cancer, the most dangerous form of UC (Wang *et al.*, 2016). As a result, persons with ulcerative colitis have a high risk of developing colon cancer. Each year, the probability of this happening grows. Compared to rural areas, these illnesses primarily affect urban and western industrialized areas (Kulaylat *et al.*, 2010).

Inflammatory bowel disease (IBD) is a term used to describe a set of chronic inflammatory diseases that affect the digestive tract and are characterized by abdominal pain and diarrhea (Fakhoury *et al.*, 2014). UC is a variant of colon cancer limited to the colon and characterized by chronic inflammation that constantly involves the rectum (Clarke *et al.*, 2019). It is classified according to its proximal limit. In UC variation CD, inflammation is limited to the mucosal surface. Figure 1.1 shows the comparison of ulcerative colitis with Crohn's disease.

Meanwhile, there are many inflammatory parallels between UC and CD, such as epithelial barrier failure, hereditary susceptibility, etc. IBD can cause severe morbidity and mortality and shorten life expectancy (Table 1.1). Regarding UC, the inflammatory response and morphologic changes are limited to the colon (Yang *et al.*, 2009).

Table 1.1: Difference between Ulcerative colitis and Crohn's disease

S. No.	Ulcerative colitis	Crohn's disease
1.	Patients with ulcerative colitis have 10 fold increased risk	High prevalence in Ashkejews according to disease frequency
2.	Jewish families with UC are at higher risk than non-Jewish families	Familial traits/ aggregation is more evident
3.	Significant histocompatibility complex class II allelic connections are more adverse for UC than Crohn's disease	The harmonical rate for monozygotic twins is approximately 44%, which is more than dizygotic twins having 3-8%
4.	DRB1*1502, DRB1*0103 and DRB1*12 genes show positive association, while DR4 and Drw6 show a negative association	Expression of the NOD2 gene in monocytes shows more frequent mutations in patients with Crohn's disease than in UC
5.	MUC3 gene encodes intestinal mucins are associated with the pathogenesis of UC	NOD2 variants, CD11 integrins, CD19 sialophorin, and IL-4 receptors are involved in the pathogenesis of Crohn's disease

In 95% of cases, the rectum is affected, with varying degrees of proximal extension. Only the innermost lining or mucosa is affected, resulting in continuous zones of inflammation, ulceration, edema, and bleeding down the colon's length (Goyal *et al.*, 2014). The presence of blood and mucus fused with feces is the most persistent feature of UC, accompanied by lower abdominal pain that is most severe during bowel movements. Aminosalicylates, glucocorticoids, immunomodulators, and other conventional and unconventional treatments for UC include aminosalicylates,

glucocorticoids, and immunomodulators (Harrington *et al.*, 2020).

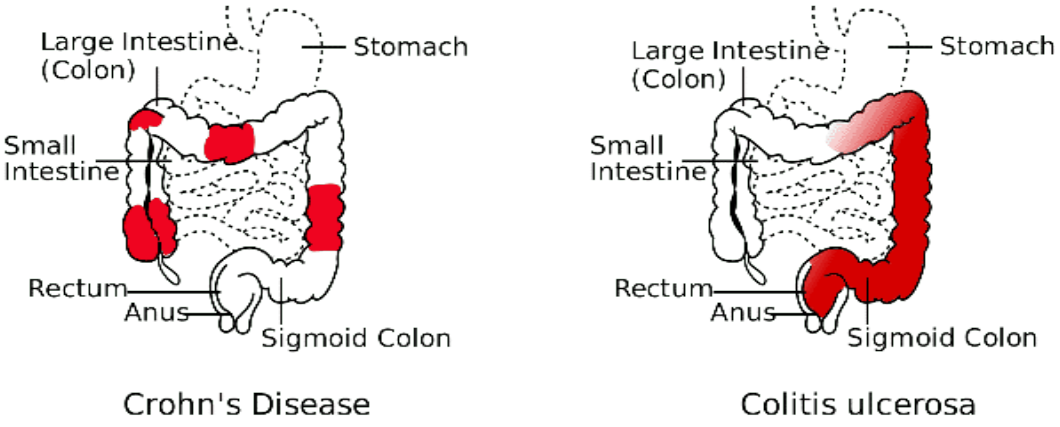


Figure 1.1: Comparison of ulcerative colitis with Crohn's disease.

These should be given to patients regularly, which reduces patient compliance and can result in systemic side effects. As a result, oral medication delivery has been discovered to be an advantageous method for regulated and targeted drug delivery, reducing dosage frequency and improving patient compliance. Compared to Crohn's disease, which does not present bloody diarrhea, the prominent early symptoms of ulcerative colitis are stomach pain, tenesmus, anorexia, and bloody or mucous diarrhea (Nascimento *et al.*, 2020). Weight loss, tachycardia, fever, and anemia are all signs of ulcerative colitis in its most severe forms (Figure 1.2). In the interim, it's usually associated with complete symptom remission. Both the male and female sexes are affected in the same way. UC is classified into four types based on the degree of the disease: extensive, distal, proctosigmoiditis, and ulcerative proctitis (Paine *et al.*, 2014).

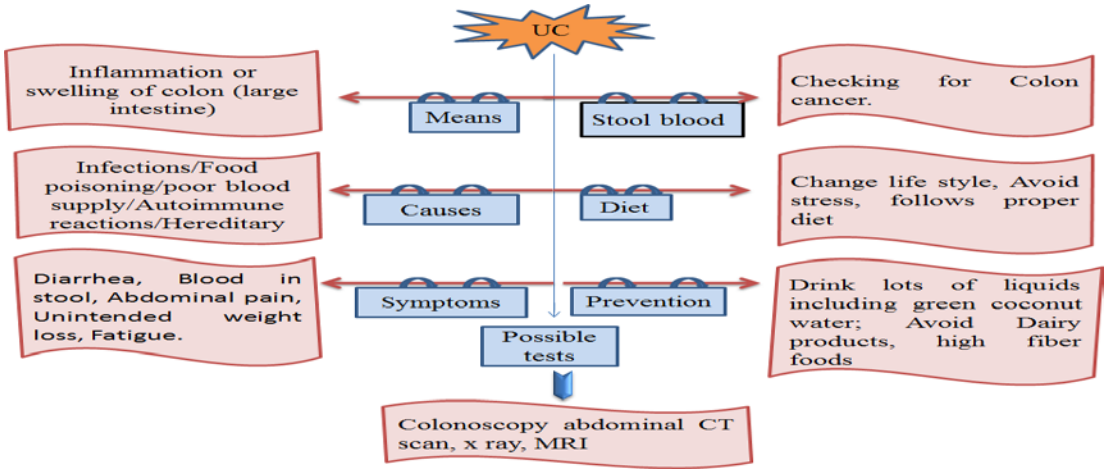


Figure 1.2: Representation of schematic flow chart that specifies ulcerative colitis.

1.2 EPIDEMIOLOGY

The occurrence and prevalence of UC have been expanding over time worldwide. This disease affects around 24.3 per 100,000 people in Western Europe, 6.3 per 100,000 people in Asia, and 19.2 per 100,000 North Americans yearly (Rashid *et al.*, 2016). Because of its rising occurrence and prevalence over time, IBD is becoming a global condition. The majority in Africa, South America, and Asia are relatively low. In the United States, it is estimated that over 1 million people suffer from IBD, with 100,000 of them being children. Among the most prevalent gastrointestinal disorders, IBD is ranked 5th (Loftus *et al.*, 2016).

UC is mainly related to recurrent attacks with complete remission of symptoms. The increased incidence of the disease is more (three to six-fold more) in Jewish and it is more common in Caucasians than in black (Kaplan *et al.*, 2017).

In Western Europe, the occurrence of approximately 6 to 8 cases per 100,000 populations and in the USA, an estimated occurrence of about 70 to 150 per 100,000 populations. Europe seems to have a variance in UC incidence; eastern countries have lo incidence than western and northern countries. The chances of an immigrant's child developing ulcerative colitis and progressing from a low-to-high-occurrence condition are the same for immigrant children. IBD is becoming a significant problem

worldwide as the incidence and spread of the disease increase over time (Windsor *et al.*, 2019).

1.3 ETIOLOGY

Although the origin of UC is unknown, some factors are linked to the disease, including family history, oral contraceptive usage, genetics, Gut/environmental, psychological, autoimmune, and epidemiological factors. UC is an autoimmune disease triggered by colonic bacteria and causes gastrointestinal system inflammation (Torres *et al.*, 2012). Around 25-40% of children have a family history of chrons disease; however, someone with chrons disease is 17-35 times more likely to acquire ulcerative colitis than the general population. CD may be caused by a combination of causes, including aberrant mucosal immune responses, intestinal epithelial dysfunction, and problems in host-microbe interactions (Larabi *et al.*, 2020).

Environmental factors include immune interactions, bacterial infections, and epithelial barrier functions. Healthy behavior, smoking habits, drug consumption, hormonal conditions, temperature fluctuations, and changes owing to social situations are all studied in epidemiological studies (Figure 1.3). According to hygiene theory, the drop in enteric infections in wealthy countries has resulted in insufficient progress in the regulatory mechanisms responsible for mucosal immune responses (Chen *et al.*, 2019; Vancamelbeke *et al.*, 2017).

Different cell signal pathways, inflammatory mediators such as tumor necrosis factor (TNFα), Interleukin-6, Interleukin-1, Interleukin-4, Interleukin-12, Interleukin-11, and Interleukin-4, and Eicosanoids profiles can all be used to analyze inflammatory Factors. Dysplasia, caused by chronic inflammation caused by reactive oxygen species, can lead to CAC, or colitis-associated colorectal cancer, a severe kind of ulcerative colitis (Kany *et al.*, 2019).

As a result, individuals with UC have a high risk of developing colon cancer. The mucosal layer of the colon is where the inflammation is kept contained. The rectum is

always implicated, with inflammation spreading proximally synchronously. In contrast, the CD is not bound to a specific area and can occur in any section of the GIT, with inflammation that can be asymmetrical, transmural, or segmental (Hirsch *et al.*, 2021).

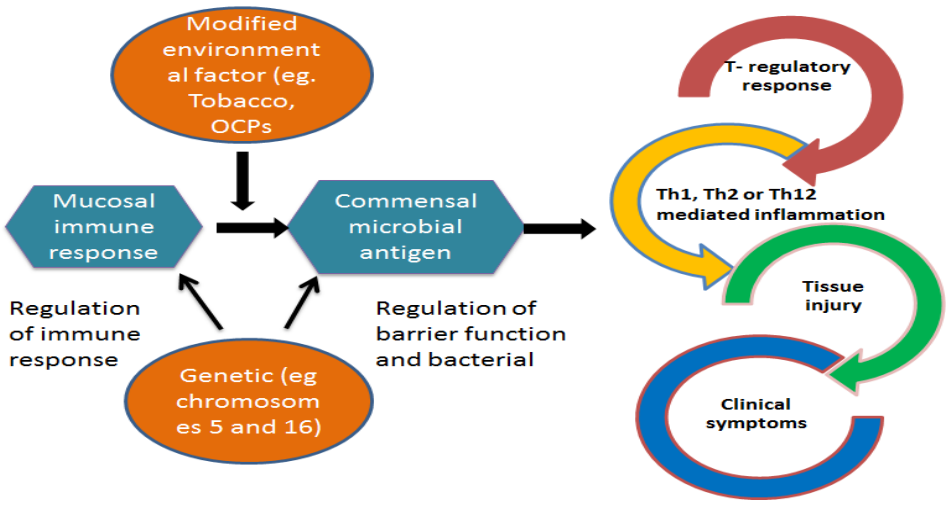


Figure 1.3: Etiologic factors in IBD.

1.4 FACTORS RESPONSIBLE FOR ULCERATIVE COLITIS

1.4.1 Genetic factors: There's much evidence that genetic susceptibility plays a more significant role in Crohn's disease than UC. Immunogenetics has long been thought to play a role in the tendency to UC, with several susceptibility genes. The two primary genes, NOD1 and NOD2, are the expression genes that activate nuclear factor kappa B (NF-κB), which is involved in the start of inflammatory responses and includes tumor necrosis factor (TNF α), Interleukin-1, IL-6, and IL-1. Table 2.2 shows variable genetic factors involved in the pathogenesis of IBD (Ahmad *et al.*, 2006).

1.4.2 Environmental factors

Environmental factors have an unclear role in UC growth. Over the last 50 years, the frequency of CR disease has increased dramatically in industrialized countries

compared to developing countries (Cui *et al.*, 2018).

When the environment changes, the odds of developing a deregulated immune system and an increase or decrease in intestinal microbiota. The genes that give mucosal immune protection become increasingly sensitive due to modern lifestyle changes, and they begin to provide increased mucosal immunological responsiveness (Legaki *et al.*, 2016). Other factors, such as cigarette smoking, increased intestinal permeability, and psychological stress, has been linked to UC exacerbation (Table 1.2). Appendectomy has also been linked to UC exacerbation. Non-smoking has been related to UC and has been shown to protect patients in previous research. Although nicotine is the primary pharmacological substance responsible for smoking's effects, its specific mechanism is unknown (Perricone *et al.*, 2016). According to several studies, nicotine inhibits the synthesis of proinflammatory cytokines such as interleukin-1, TNFα, and IL-8 and promotes colonic mucus formation, which impacts cellular and humoral immunity.

Table 1.2: Environmental factors involved with the pathogenesis of IBD

S.No.	Ulcerative colitis	Crohn's disease
1.	Smoking protects patients with UC	The negative effect of smoking in patients with Crohn's disease
2.	NSAIDs exacerbate ulcerative colitis	People living in different geographical areas show variations in risk for disease
3.	Psychological stress is the leading cause of UC	Improved sanitation
4.	Appendicectomy, at a young age, protects against the development of UC	Utilization of non-fermented and sterile food

1.4.3 Microbial factors

In the pathophysiology of UC, microbial organisms play a crucial role. Mycobacterium paratuberculosis, measles virus, and listeria monocytogenes have all been implicated in IBD for decades (Table 1.3). Current research shows that UC is linked to a significant decrease in anaerobic bacteria and *Lactobacillus* (Hindryckx *et al.*, 2016).

Table 1.3: Microbial factors linked with the pathogenesis of UC

S.No.	Ulcerative colitis	Crohn's disease
1.	Colitis develops due to higher exposure of bacteria microflora to the body but not if germ-free	Lesions develop mainly in the bowel region with the highest bacterial count.
2.	Probiotics have protective effects on pouchitis	Antibiotics produce a protective effect on humans
3.	Infections occur due to seasonal variations	Tolerance capacity has been lost due to the occurrence of immune reactions against enteric bacteria

1.4.4 Immuno-inflammatory factors

1.4.4.1CD4$^+$ Cells and T- helper cells

CD4$^+$ cells play an essential role in the control of particular immunological responses. Th1 and Th2 CD4$^+$ cells are differentiated by their ability to generate cytokines. Th1 cells regulate cell-mediated immune responses by secreting IL-2, IL-12, and interferon-, whereas Th2 cells regulate humoral immune responses by secreting IL-4, IL-5, IL-6, IL-10, and IL-13. Activated mucosal T cells are controlled by at least two mechanisms, both of which are reciprocal. Antigen-presenting cells (APCs) enable intraluminal antigens to adhere to luminal mucosal lymphocytes, resulting in the development of effector responses. In contrast to regulatory T cells,

which suppress T- effector responses, such as inflammation, effector T cells produce intestinal inflammation by producing inflammatory cytokines like IL-12 (Gagliani *et al.*, 2017; Luckheeram *et al.*, 2012).

1.5 PATHOGENESIS OF ULCERATIVE COLITIS

Although the pathogenesis of UC is uncertain, some recent results have pointed to an over- or under-regulation of the mucosal immune system as a critical pathophysiological pathway, with particular emphasis placed on immunologic reactions or mucosal inflammation (Du *et al.*, 2020).

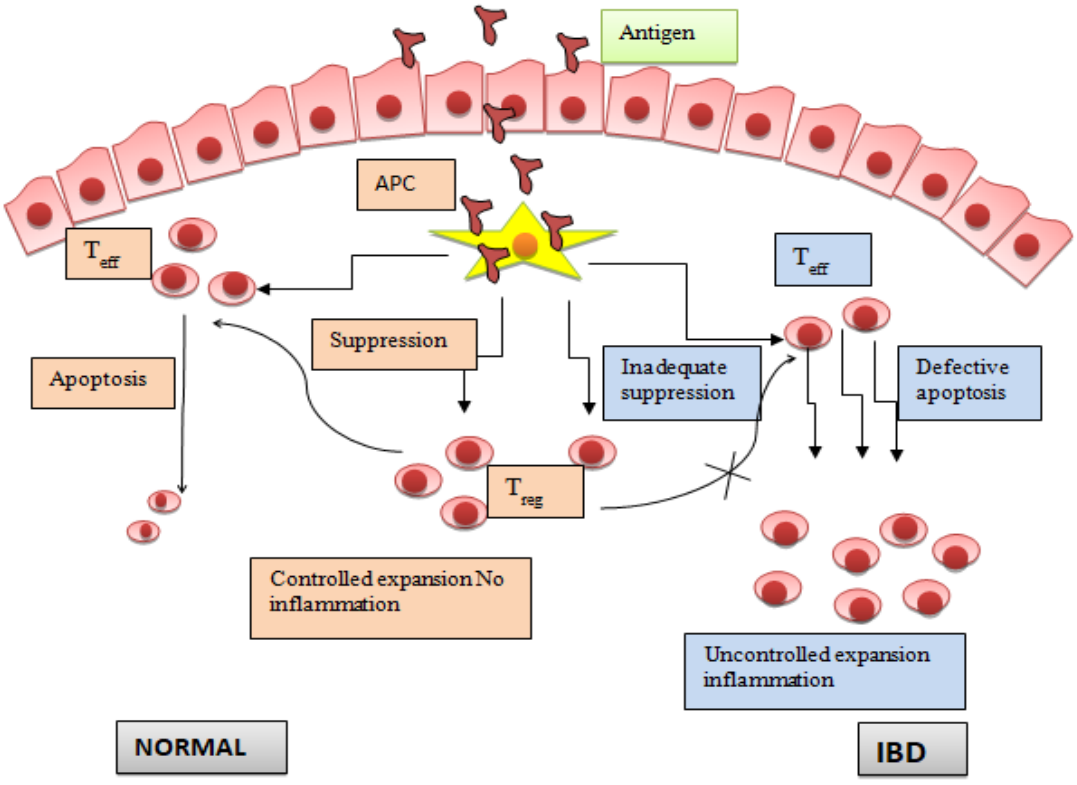


Figure 1.4: Pathogenesis of ulcerative colitis (UC) is the Intraluminal antigens to mucosal lymphocytes by antigen-presenting cells (APCs) leading to the generation of effector responses. Such as an effect of an effector response, there is an overproduction of effector T cells (Teff) that leads to inflammation by the generation of inductive cytokines such as IL-12.

Primary immunological abnormalities or an insufficient pathogenic immune response to an environment, such as commensal gut bacteria, are caused by various factors in ulcerative colitis (Figure 1.4). The immune system's dysregulation is the first significant reason which leads to uncontrolled immunological reactions to normal microbiota (Guo *et al.*, 2020).

Deregulations of the immune system emanate directly from the rectum in a continuous pattern involving part or all of the colons in most instances (i.e., 95%). A second cause is epithelial cell abnormalities and changes in gut microbiota content, both of which promote an atypical mucosal immune response (Sartor *et al.*, 2006).

Reduced gene expression, or change of the gene that is expressed, is the third cause. UC is a chronic inflammatory disease affecting the large intestine and colon, in which the entire organ or a section of the gastrointestinal tract is, afflicted (Hanauer *et al.*, 2004).

UC is an inflammatory bowel disease characterized by continuous inflammation and ulceration that extends from the rectum to the caecum and is usually associated with increased IL-13 production, whereas Crohn's disease is associated with increased IL-12/IL-23 and IFN-/IL-17 production. It typically affects the ileum and colon, with intermittent ulceration and inflammation, including granulomas. Initiation of NF-κB can also lead to ulcerative colitis. In its natural state, NF-κB is found in the cytoplasm, where it attaches to I-κB and regulates the immune system (Di Sabatino *et al.*, 2012).

However, in ulcerative colitis, antigen stimulates NF-κB, and phosphorylase I-κB leads to proteasome breakdown. When NF-κB is liberated, it can go to the nucleus and trigger the transcription of proinflammatory genes, including IL-6, IL-1, and TNF-, resulting in immunological dysregulation.

Figure 1.5 shows the mechanism of ulcerative colitis. According to some experts, one of the causes that create ulcerative colitis and result in disease continuation is abnormally elevated intestinal permeability. By triggering the inflammatory process, the barrier function of epithelial wall conduct is broken, resulting in increased mucosa permeability for luminal antigens, germs, or microbes, as well as loss of water and

electrolytes (Kaistha *et al.*, 2014).

Water and other electrolytes have been lost due to the broken epithelial barrier. As a result of this, injured intestinal cells lose their polarity.

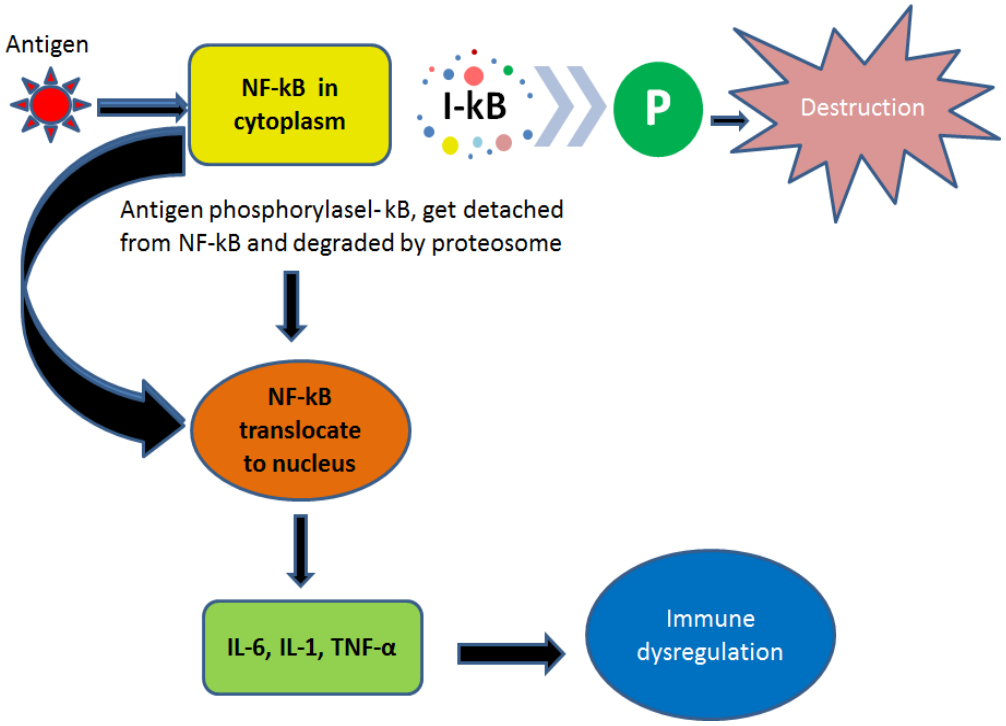


Figure 1.5: Mechanism of UC via NF-κB mechanism.

Apical expression of transferrin receptor protein is most elevated on the apical and basolateral regions of enterocytes in IBD patients' inflamed mucosa.

1.6 SIGNS AND SYMPTOMS OF ULCERATIVE COLITIS

The main symptoms of UC include pain, discomfort and diarrhea with blood (Table 1.4). Fever and weight loss occur sometimes. Extraintestinal symptoms can be an initial expression or occur subsequently during the illness (Elahi *et al.*, 2012).

Obstipation may be the first symptom of proctitis on rare occasions. Significant signs and symptoms include weight loss, tachycardia, rectal bleeding, and bowel inflation (Table 1.4). Distal, extensive, and ulcerative proctitis are different types of ulcerative colitis. Proctitis affects about 80% of patients, and 20% have severe colitis. However,

proximal extension occurs late in about half of all individuals.

Table 1.4: Initial symptoms of UC

S. No.	Symptoms	% frequency
1.	Diarrhea	96.4
2.	Blood in stool	89.3
3.	Pain	81.3
4.	Generally unwell	40.2
5.	Arthralgia	27.7
6.	Fever	20.5
7.	Weight Loss	38.4
8	Skin Changes	20.5
9	Loss of appetite	15.2
10	Ophtalmopathies	7.1

Table 1.5 : Comparison of Ulcerative colitis vs. Crohn's disease

Features	Ulcerative colitis	Crohn disease
Site of origin	Rectum	Terminal ileum
Symptoms	Bloody diarrhea	Crampy abdominal pain
Thickness of inflammation	Submucosa or mucosa	Transmural
Complication	Hemorrhage, Toxic megacolon	Fistulas, abscess, obstruction
Cytokine response	Associated with T_h17	Associated with T_h2
Distribution	Continuous distribution	Discontinuous
Drugs used	5-Aminosalicylic acid, Sulfasalazine, Balsalazide, Infliximab, Azathioprine, and mercaptopurine	Hydrocortisone, Budesonide, Prednisolone, Sulfasalazine, Olsalazine, Mesalazine

Table 1.6: Drugs used in the treatment of ulcerative colitis

Drug Type	Drug Name	Available Routes	Efficacy	Induction Dose	Maintenance Dose	Adverse Events
5-Aminosalycilate	Mesalamine Balsalazide Sulfasalazine	Oral	Induction and maintenance	Mesalamine: 2-4.8 g (oral) Balsalazide: 6.75 g Sulfasalazine: 2-4 g	Mesalamine: 1.6-2.4g Balsalazide: 6.75 g Sulfasalazine: 2-4 g	Headache, nausea, diarrhea, interstitial nephritis, leukopenia, and hepatitis
Corticosteroids	Prednisone Budesonide Methylprednisolone	Oral Rectal IV	Induction only	Prednisone: 40-60 mg Budesonide: 9 mg Methylprednisolone: 40-60 mg total daily dose	Budesonide -9 mg/day	Delirium, cataracts, glaucoma, striae, delayed wound healing
Thiopurines	Azathioprine Mercaptopurine	Oral	Induction and maintenance	Azathioprine: 2-2.5 g/kg Mercaptopurine: 1-1.5 g/kg	Azathioprine: 2-2.5 g/kg Mercaptopurine: 1-1.5 g/kg	Nausea, vomiting, hepatitis, bone marrow suppression, pancreatitis

Anti-TNF	Infliximab AdalimumabGolimumab	IV Subcutaneous	Induction and maintenance	Infliximab: 5 mg/kg weeks 0, 2, and 6 Adalimumab: 160 mg week 0, 80 mg week 2 Golimumab: 200 mg week 0, 100 mg week 2	Infliximab: 5 mg/kg every 8 weeks Adalimumab: 40 mg every 2 weeks Golimumab: 100 mg every 4 weeks	Infusion/injection site reaction, infection, melanoma, reactivation of latent TB and hepatitis B
Calcineurin inhibitor	Cyclosporine Tacrolimus	IV Oral	Induction only	Cyclosporine: 2-4 mg/kg daily (dose to trough level, 200-400 ng/mL) Tacrolimus: 0.2 mg/kg (dose to trough level, 10-15 ng/mL)	Tacrolimus: 0.2 mg/kg (dose to trough level, 10-15 ng/mL)	Hypertension, nephrotoxicity, hyperkalemia, infection, lymphoma, skin cancer
Adhesion molecule inhibitor	Vedolizumab	IV	Induction and maintenance	300 mg weeks 0, 2, and 6	300 mg every 8 weeks	Infusion reactions, infection

With proctosigmoiditis, and the opposite occurs in the other half, a shift in the disorder's location should result in new symptoms. The length of the illness can vary (Singh *et al.*, 2020).

1.7 CURRENTLY USED DRUGS THERAPY IN THE MANAGEMENT OF ULCERATIVE COLITIS

Anti-inflammatory medicines, which generally include 5-aminosalicylates like olsalazine, mesalazine, and balsalazide, are the most commonly used to cure and manage UC (Table 1.6). They can treat mild to moderate episodes and can maintain remission in UC. Azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus, and calcineurin inhibitors are examples of immunosuppressive drugs. Corticosteroids, such as prednisolone and anti-TNF-antibodies, are two different types of medicines used to treat IBD, which range from mild to severe. Surgery may be a preferable alternative for IBD patients in the refractory and fulminate illness phases (Alsoud *et al.*, 2021, Sinopoulou *et al.*, 2021, Hirten *et al.*, 2021, Xu *et al.*, 2004, Gionchetti *et al.*, 2003).

1.8 MESALAMINE AS A POTENT ANTIOXIDANT AND ANTI-INFLAMMATORY DRUG

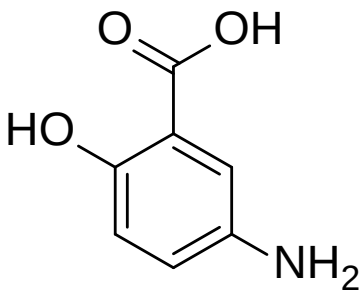
1.8.1 Introduction of Mesalamine

Chemical name: 5-amino-2-hydroxybenzoic acid

Empirical formula: $C_7H_7NO_3$

Molecular weight: 153.14g/mol.

Structural formula:



Appearances: Mesalamine is a white powder and organoleptic character.

Melting point: 282-283 ^{0}C

Solubility: Water Solubility (0.84 g/L at 20^{0}C), practically insoluble in acetone and methanol soluble in DMSO.

Brand names: Asacol, Asacolitin, Canasa, Claversal, Fisalamine, Lixacol, Mesasal, Pentasa, Rowasa, Salofalk

Half-life: The mean elimination half-life was 5 hours for 5-ASA and six hours for N-acetyl-5-ASA following the initial dose. At a steady state, the mean elimination half-life was seven hours for both 5-ASA and N- acetyl-5-ASA.

Mechanism of action - Although the mechanism of action of Mesalamine is not fully understood, it appears to be topical rather than systemic. Mucosal production of arachidonic acid metabolites, both through the cyclooxygenase pathways, i.e., prostanoids, and through the lipoxygenase pathways, i.e., leukotrienes and hydroxyeicosatetraenoic acids, is increased in patients with chronic inflammatory bowel disease, and Mesalamine may diminish inflammation by blocking cyclooxygenase and inhibiting prostaglandin production in the colon.

Pharmacology: Mesalamine is an anti-inflammatory drug used to treat digestive tract inflammation and mild to moderate ulcerative colitis. Mesalamine is a bowel-specific aminosalicylate drug metabolized in the gut and has its predominant actions, thereby having fewer systemic side effects. As a derivative of salicylic acid, 5-ASA is also an antioxidant that traps free radicals, potentially damaging by-products of metabolism

(Rizzello *et al.*, 2019). In UC, reactive oxygen species and inflammatory mediators played critical; Mesalamine decreases the reactive oxygen species formation and the concentration of inflammation mediators (Managlia *et al.*, 2013).

The previously published data suggested that Mesalamine is a good drug for managing UC. In the conventional treatment of UC, Mesalamine is the first-line choice of medication available in tablets (Mesacol), capsules (Pentasa), and rectal enemas (Perrigo). By the oral route, it is rapidly absorbed from the small intestine and fails to reach the inflamed colonic site. Furthermore, long-term medication produces many side effects, negatively affecting the quality of life of UC suffered patients. Additionally, poor targeting of the orally administered drug to the desired site represents frequent therapeutic challenges (Tripathi *et al.*, 2019).

1.9 PROBIOTICS' ROLE IN ULCERATIVE COLITIS

Probiotics are live microorganisms that provide health welfare to the host when given an indefinite quantity. The colon contains over 400 distinct species of bacteria having a population of 10^{11}–10^{12} CFU (Colony Forming Unit) ml^{-1} with *Bacteroides, Bifidobacterium, Eubacterium* and *Lactobacillus* considerably outnumbering other species (Table 1.7). UC is a mucosal disease; probiotic therapy, which works on the mucosal level, might be beneficial. Probiotics act by several mechanisms. Firstly, they act as a safety barrier, covering the intestinal tract near the brush border and through competitive inhibition. They do not allow the luminal bacteria from reaching the lamina propria and stimulating the mucosal immune system. Secondly, probiotics enhance mucous production and can modify the mucus's consistency, thus changing bacterial adherence patterns and protecting against bacteria. Thirdly, probiotics cause the mucosal immune system to secrete the protective immunoglobulin (Ig) such as secretory IgA changes the function of the mucosal immune system to make it more anti-inflammatory (Zipporah *et al.*, 2019) and a host of protective bacteriocins and defensins into the lumen.

This mechanism appears to be particularly important in UC. With all these mechanisms, probiotics can diminish the effect of luminal bacteria responsible for inflammation. Administration of probiotics may recover the commensal microflora and normalize the host-microbial interaction. Certain types of bacteria have probiotic properties. They essentially alter the type and amount of bacteria in the gastrointestinal tract and positively manipulate the enteric flora. They have local and systemic effects, such as regulating the host immune response and improving the epithelial barrier function by decreasing intestinal permeability and enhancing tight junctions. Probiotics work for a healthier gut by indirectly improving the number of fatty acids with short chains, which lower colonic pH and thus help stop the development of pathogenic bacteria. The short-chain fatty acids butyrate provides nutrition to the intestinal epithelium. Co-administration of probiotics along with the drug delivery system refurbishes the colonic micro-flora damaged or lost due to the effect of the drug as well as pathophysiological conditions in UC (Floch *et al.*, 2018). In addition to strain and species, mechanisms are also dependent on factors such as

Table 1.7: Microorganisms commonly used as probiotics

LACTOBACILLI	BIFIDOBACTERIA	OTHERS
L. casei Shirota	*B. longum*	*Escherichia coli Nissle*
L. rhamnosus	*B. bifidum*	*Saccharomyces boulardii*
L. johnsonii	*B. infanitis*	*Enterococcus faecalis*
L. acidophilus	*B. lactis*	*Lactococcuslactis*
L. gasseri	*B. breve*	*Propionibacteria*
L. reuteri	*B. animalis*	
L. casei	*B. adolescentis*	
L. fermentum		
L. cripatus		

bacterial environment and the disease setting under investigation. Common mechanisms of action identified in probiotics include inhibition of pathogenic enteric bacteria, improvement of epithelial barrier function, and manipulation of host immunoregulation. The research on probiotics today aims to characterize the microbiota in each individual, analyzing the species constitution and the number of different bacteria in the intestine (Agraib *et al.*, 2021).

1.9.1 *L. acidophilus*

L. acidophilus is a gram-positive bacteria species belonging to the *Lactobacillus genus. L. acidophilus* is a homofermentative, microaerophilic species that ferments carbohydrates into lactic acid. It grows well at low pH levels (below pH 5.0) and a temperature of around 37°C. from the literature survey, it has been concluded that the *L. acidophilus* probiotic, combined with olsalazine, could be suggested as adjuvant therapy for ulcerative colitis treatment (Dhillon *et al.*, 2020; Prisciandaro *et al.*, 2009; Amer *et al.*, 2018; Celiberto *et al.*, 2017; Serban *et al.*, 2015).

1.9.2 *S.boulardii*

S. boulardii is tropical yeast first isolated from the fruit of lychee and mangosteen. *S. boulardii* is a *S. cerevisiae* strain. It grows at 37°C. It is a nonpathogenic strain of yeast. *S. boulardii* has probiotic properties and is also the only yeast approved as a human probiotic. Several controlled studies show that *S. boulardii* has a positive effect on UC (Sivananthan *et al.*, 2018; Sheil B *et al.*, 2007; Cain *et al.*, 2011; Isaacs K *et al.*, 2008).

1.10 CHALLENGES IN THE TREATMENT OF ULCERATIVE COLITIS

The significant challenges in the therapy of ulcerative colitis are the reduction of drug-related side-effects, i.e., weight loss, rectal bleeding, anemia, tachycardia, and bowel distension by drug delivery to the colon is site-specific (Fukuda *et al.*, 2019).

Also, long-term medication produces many harmful side effects, which negatively affect the value of the life of IBD patients. Such a delivery system delivers the maximum amount of drug to the specific site at the right time in the body, increasing efficacy (Kane *et al.*, 2008).

Several agents are taken for the therapy of ulcerative colitis diseases, but they show adverse effects like diarrhea, peptic ulcers, nephron and hepatotoxicity, vomiting, glaucoma, Cushing's syndrome, etc. (Lin *et al.*, 2017).

Targeting the drugs, particularly to the colonic part of GIT, is the main challenge. Synthetic drugs have various drawbacks, so safe and efficacious drug treatment for the UC is the problem (Lee-Kong *et al.*, 2016).To solve these difficulties, appropriate anti-inflammatory therapy is the best option. Drawbacks in the current therapy include:

- Systemic toxicity
- Higher dosing
- Non-specific drug delivery
- Adverse drug effects

1.11 STRATEGIES FOR THE MANAGEMENT OF ULCERATIVE COLITIS

1.11.1 Microparticles as carrier

Microparticles are used for the targeted drug delivery type to manage ulcerative colitis. Microparticles are suitable for various drug delivery applications and offer many advantages (Singh *et al.*, 2014; Lee *et al.*, 2020).

- Their small particle size offers improved reproducibility in the drug release mechanism.
- They offer improved control over the release rate of the drug.
- A microparticle offers immediate, modified, delayed, pulsed, sustained and

extended-release.

- They allow the preparation of dosage forms for colon delivery through the coatings designed.
- Their reduced particle size gives them the potential to improve bioavailability.

The primary motive of this delivery system is to target the high concentration of active ingredients at the site in the inflamed intestinal tissues to enhance the therapeutic efficacy and minimize the side effect. Microparticle systems provide a selective drug targeting the specific location with a loss in the needed efficacious drug dose and side effects. So the formation of a novel site-specific drug delivery system that will increase the drug release in the inflamed tissues without causing any harm to normal tissues and then decrease the side effects of the drug is needed (Lengyel *et al.*, 2019).

The primary step in this direction is preparing pharmaceutical dosage forms with reduced sizes, which will enhance the time of their residence in the colonic part. The most common characteristic of IBD is diarrhea that causes streaming of dosage form (rapid transit of large dosage forms (Chaudhari *et al.*, 2017).

The retention time can be enhanced by decreasing the size of dosage forms (e.g., pellets). Further, a decrease in size to the micrometer range will help to reduce the streaming effect (and thereby increase the residence time) and also helps in enhancing the bio-distribution of the drug molecule. Lately, many innovative ideas have been explored for the management of IBD. The main motive behind developing these targeted drug delivery systems was to reach site-specific transport of active moieties to the inflamed tissue (Jain *et al.*, 2020).

These drug carrier systems not only prevent the degradation of active moieties against various physiological changes during IBD but also increase the therapeutic effectiveness and lessen systemic adverse drug reactions. Research and development in the treatment of IBD are observing steady-state progress in developing upgraded

and smart drug delivery systems and highly effective therapeutic agents. One can say that attaining effective and site-specific targeting for the treatment of IBD will soon be able to knock on the doors of reality (Lamprecht *et al.*, 2003).

The mechanism of uptake of nanocarriers/ microcarriers for site-specific drug delivery depends upon the complete information of the means of disease and drug. Some studies have stated that for ulcerative colitis treatment, the microparticle range should be 10–300 µm to target specifically the inflamed region of the colon (Collnot *et al.*, 2012).

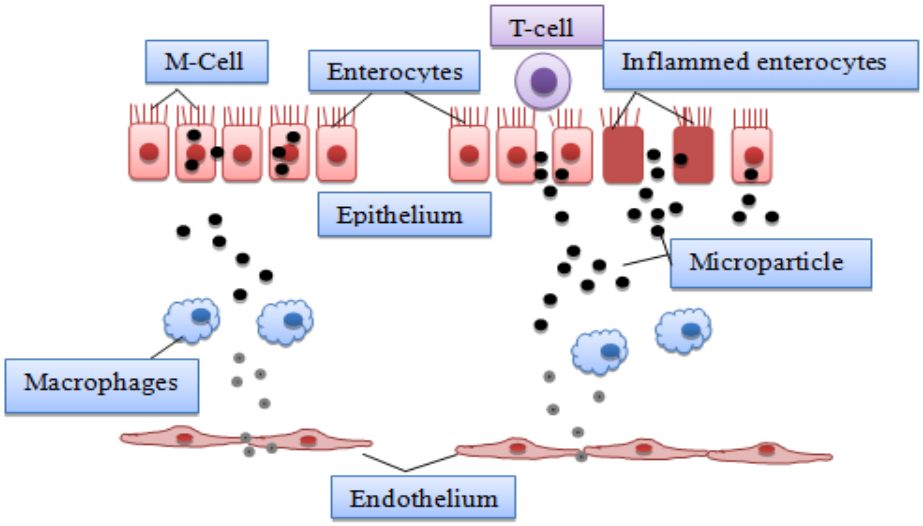


Figure 1.6: Particle uptake in uninflamed and inflamed mucosa.

Carrier selection is essential for a particular drug, i.e., either hydrophilic or lipophilic and also depends on the disordered situations and the physicochemical nature of the drug. The optimal particle size should be between 4 to 15µm for improved localization and increased drug residence time at the site of inflammation (Glavas-Dodov *et al.*, 2013).

To attain high localization in Payer's patches, intestinal lymphoid tissue and lamina propria, there is the need to overcome the G.I.T. barrier/layer. In the case of UC, as the disease severity increases, the protective mucus layer becomes thinner. This

pathophysiology of the mucus layer increases the mucosal permeability and helps in the proper location at its inflamed sites. Size-dependent translocation of MP and NP across colonocytes in healthy GI tract contains the following processes that are shown in Figure 1.6

1) Particles of size range < 500 nm show endocytotic uptake through endocytosis.

2) Particles of size <5 µm adsorbed by M cells of Peyer's patches show lymphatic uptake.

3) Using mucoadhesive coating polymers increases the bioadhesion of microparticles/ nanoparticles (Sosnik *et al.*, 2014).

1.11.2 Pellets as a carrier

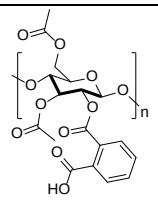
Pellets are solid, small free flowing spherical units prepared by agglomeration of fine powder called pellets. Pellets range in size typically between 0.5 – 1.5 mm, though other sizes could be prepared. Bulking agents and binders are required to prepare pellets by extrusion and spheronization methods. We use pectin as a core material binder agent and MCC as a bulking agent. CAP as a coating polymer for colon-targeted drug delivery (Singh *et al.*, 2010).

1.12 POLYMER SELECTION

1.12.1 Profile of Cellulose Acetate Phthalate (CAP)

Cellulose acetate phthalate (CAP): Cellulose acetate phthalate is a reaction product of phthalic anhydride and a partial acetate ester of cellulose. On an anhydrous acid-free basis, it comprises 21.5–26.0% acetyl (C_2H_3O) groups and 30.0-36.0% phthalate (o-carboxybenzoyl, $C_8H_5O_3$) groups. CAP is a commonly used polymer phthalate in the formulation of pharmaceuticals, such as the coating of tablets or capsules and for controlled release formulations and an enteric-coated polymer (Table 1.8). It prevents the release of drugs from an acidic environment (Kamel *et al.*, 2008; Marques-Marinho *et al.*, 2013; Verma *et al.*, 2012; Bardoliwala *et al.*, 2021).

Table 1.8: Profile of CAP

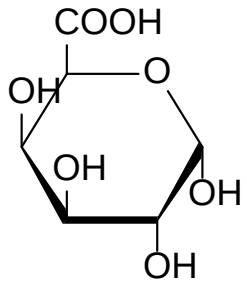
Chemical name	Acetate, 1,2-benzenedicarboxylate
Molecular formula	$C_{156}H_{174}O_{95}$
Molecular structure	
Molar mass	3569.013
Appearance	White to off-white solid
Odor	A slight odor of acetic acid
Color	White to off-white
Solubility	Freely soluble in the mildly primary medium of the intestine
Toxicity to animals	High doses of cellulose acetate phthalate in the diet tended to produce a mucilaginous character of the material in the intestinal lumen

1.12.2 Enzyme-based polymer

Pectin: Pectin, a natural heteropolysaccharide, is characterized with 1, 4 linked a-D-galactosyluronic acid residues and neutral sugars such as rhamnose, galactose and arabinose (Table 1.9). It is popularly used as a colon- a specific drug delivery vehicle for colon disease. It has a positive effect on ulcerative colitis disease state. Pectin can be selectively digested by colonic microflora to induce site-specific drug release and has a strong tendency to interrupt colon tumor growth biochemically (Noreen *et al.*, 2017).

The pectin is an aqueous soluble polymer and it undergoes partial degradation at gastric pH 2–4 via side chain hydrolysis and at small intestinal pH 5–6 via b-elimination of main chain or de-esterification. The matrix of pectin tends to swell,

Table 1.9: Profile of Pectin

Chemical name	β-D-galactopyranuronic acid
Molecular formula	$C_6H_{10}O_7$
Molecular structure	
Molar mass	194.14
Appearance	Amorphous
Odor	Virtually no odor
Color	White to light brown powder,
Melting point	142-144 °C
Solubility	Soluble in warm water, insoluble in chloroform and ethanol

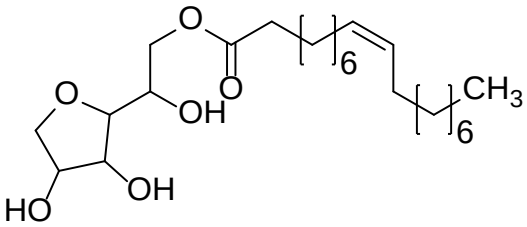
erode and has premature drug release at the upper gastrointestinal tract (Shukla *et al.*, 2012; Sriamornsak *et al.*, 2011; Niu *et al.*, 2021).

1.13 EXCIPIENTS PROFILE

1.13.1 Span 80

Span 80 is a biodegradable surfactant made from oleic acid, a natural fatty acid, and sorbitol, a sugar alcohol (Table 1.10). This sorbitan ester is very excellent in forming oil-in-water emulsions. Span 80 is a non-ionic, easy-to-handle surfactant that may be used as a w/o emulsifier and o/w co-emulsifier and pigment dispersant in non-polar liquids (Sagiri *et al.*, 2012).

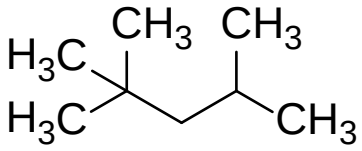
Table 1.10: Profile of Span 80

Chemical name	Sorbitan monooleate
Molecular formula	$C_{24}H_{44}O_6$
Molecular structure	
Molar mass	428.62 g/mol
Appearance	Pale yellow
Odor	Characteristic
Color	Brownish-yellow
HLB value	4.3
Density	0.986 g/mL
Solubility	Soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene

1.13.2 Iso-octane

The alkane group's iso-octane is a branching hydrocarbon. Of the eighteen octane isomers, it is the most essential. It is manufactured in a lab. Like all alkanes (paraffin, unsaturated hydrocarbons), Iso-octane is an excellent solvent for non-polar materials, fats and oils (Table 1.11). It is an inert solvent for purifying, recrystallizing, and washing active medicinal compounds, particularly in the pharmaceutical sector (Chen *et al.*, 2013).

Table 1.11: Profile of Isooctane

Chemical name	2,2,4-Trimethylpentane
Molecular formula	C_8H_{18}
Molecular structure	
Molar mass	114.23 g/mol
Odor	a petroleum-like odor
Color	Colorless liquid
Solubility	Insoluble in water

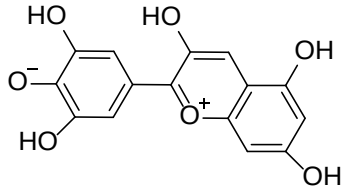
1.13.3 Light Liquid Paraffin

Light liquid paraffin oil is a highly refined mixture of liquid-saturated hydrocarbons derived from petroleum with a high paraffinic character. When chilled, it is colorless, tasteless, and odorless. The highly refined hydro-treated light liquid paraffin oil has good thermal and chemical stability and a high flash point (Table 1.12). It produces pharmaceuticals and cosmetics like creams, lotions, and perfumery (Rawlings *et al.*, 2012).

1.13.4 Microcrystalline cellulose (MCC)

MCC is a direct compression type material with good flow properties and unique diluents producing cohesive compacts. As it has high binding capabilities and offers the essential flexibility to the wet mass for successful extrusion and spheronization, microcrystalline cellulose (MCC) is the most extensively utilized excipient for pellets prepared via extrusion/spheronization (Table 1.13) (Zhao *et al.*, 2022).

Table 1.12: Profile of Light Liquid Paraffin

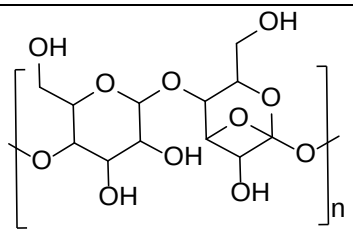
Chemical name	Liquid saturated hydrocarbons
Molecular formula	C_nH_{2n+2}
Molecular structure	
Molar mass	338.69664
Appearance	Liquid
Odor	Odourless
Color	White
Density	0.85 g/mL at 20 °C
HLB value	11 ± 1
Solubility	Soluble in chloroform, in ether and in light petroleum (boiling range 40°c to 60°c); practically insoluble in water and in ethanol (95%). Miscible with fixed and volatile oils

1.14 NEED FOR COLON-SPECIFIC DRUG DELIVERY SYSTEM

The colon has been widely investigated as a target site for drug delivery for topical treatment of local diseases (e.g., Ulcerative colitis, Crohn's disease and irritable bowel syndrome). The harsh pH conditions in the stomach and the intestinal enzymatic metabolism limit drug availability for systemic absorption and result in low bioavailability of proteins or poorly permeable drugs (Mehta *et al.*, 2011).

Compared to the stomach and small intestine, the colon has a near-neutral pH, reduced digestive enzyme activity, susceptibility to absorption enhancers and long

Table 1.13: Profile Microcrystalline cellulose

Chemical name	Cellulose
Molecular formula	$(C_6H_{10}O_5)_n$
Molecular structure	
Molar mass	342.297
Appearance	Free-flowing crystalline powder
Odor	Odorless
Color	white or almost white
Solubility	Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution

transit time (from 6 to 48hrs). These features have been taken into account to improve the oral bioavailability of some active molecules (e.g., Insulin, salmon calcitonin) by developing oral formulations which prevent drug release in the stomach and small intestine but allow drug release upon arrival in the colon (Varum *et al.*, 2008; Wang *et al.*, 2020).

Most colon-specific drug delivery systems described in the literature are based on four primary strategies: 1) Time-dependent systems, and 2) pH-dependent systems are the most widely used commercially.

1.14.1 pH-dependent system

The pH-dependent system utilizes the GI tract's pH changes to control drug release. In normal healthy individuals, there is an increase in pH from the duodenum (pH 6.6 ± 0.5) to the terminal ileum (pH 7.5 ± 0.4) and a decrease in the caecum (pH 6.4 ± 0.4),

then a slow rise from right to the left colon with final value (pH 7.0 ± 0.7) (Ibekwe *et al.*, 2008).

Coating made of pH-responsive polymers, such as Eudragit L 100-55, Eudragit S-100, CAP (Cellulose acetate phthalates), CAP (cellulose acetate phthalate), HPMCP 50 and 55, (Hydroxy propyl methyl cellulose phthalate), have been widely used alone or together in the past for site-specific drug delivery to the lower GI tract (Dai *et al.*, 2008). These polymers have a high threshold pH (at least 5.0) for dissolution and can withstand gastric acid and small intestine fluid for several hours to protect the drug from exposure to these conditions. Some examples of this delivery system are 5-Aminosalicylic acid coated with Eudragit S- 100, marketed as an Asacol tablet has been prepared for its pH-dependent (Liu *et al.*, 2017; Maroni *et al.*, 2017).

Lialda, a locally acting delayed-release tablet under the common name Mesalamine, was approved in January 2007, in which the drug is completely dispersed in the lipophilic matrix (Rashid *et al.*, 2016).

According to another study by Makham and Vakhshouri, 2010, 5-aminosalicylic acid-loaded methacrylic acid/perlite composites have been prepared, showing pH-dependent release (Mahkam *et al.*, 2010). Some other marketed formulations of multiparticulate pH-dependent systems are commercially available such as Budenofalk and Entocort. In budenofalk, pH-dependent polymers like Eudragit RL, Eudragit S, Eudragit L etc. were used whereas, in Entocort, delayed-release polymers like ethylcellulose were used for time-dependent release But, due to the lack of site-specificity of pH-dependent polymers in the colon, there exists a controversy for the reliability of the targeting method (Bagan *et al.*, 2012).

To remove such limitation, combining two polymers i.e., one is delayed-release/ time controlled and the other is pH-dependent so that the maximum amount of drug is released in the colon and shows its therapeutic effect. Fahima *et al.*, 2013, combined both time and pH-dependent systems for targeting prednisolone microspheres in the colon. He found that Eudragit S-100, an enteric-coated polymer, starts dissolving at a

pH of more than 6, which causes the lack of site-specificity of the drug at the colonic region. Therefore, he combined both Eudragit S-100 and ethylcellulose, as only 20% drug is released in the small intestine while due to delayed-release polymer, the rest 80% will release in the colon (Hashem *et al.*, 2013).

1.14.2 Microflora-activated systems

Microflora-activated systems usually refer to colon-specific carriers based on polysaccharides that bacteria can digest in the colon. The enormous population (about 10^{11}-10^{12} CFU/ml) of colon bacteria produces a range of enzymes to act on specific substrates left undigested after passage through the small intestine (Kotla *et al.*, 2014). The enzymes present a more reliable feature of the colon for achieving site-specific drug delivery when compared to transit time or pH change. Substrates for these enzymes include polysaccharides such as chitosan, amylose, cyclodextrins, pectin, guar gum and alginate, which have been used as coating or matrices in delivery systems (Asgari *et al.*, 2020).

Degrading these polysaccharides by enzymes produced by microflora in the colon subsequently activates drug release. Carrier systems combining pH-dependent and microflora-activated mechanisms have been developed to prevent burst drug release in the stomach and small intestine and achieve improved colonic delivery. Mesalamine may cause the following side effects:

- Nausea
- Diarrhea
- Abdominal pain
- Headache
- Rashes
- Hypersensitivity reactions.

1.15 RESEARCH ENVISAGED

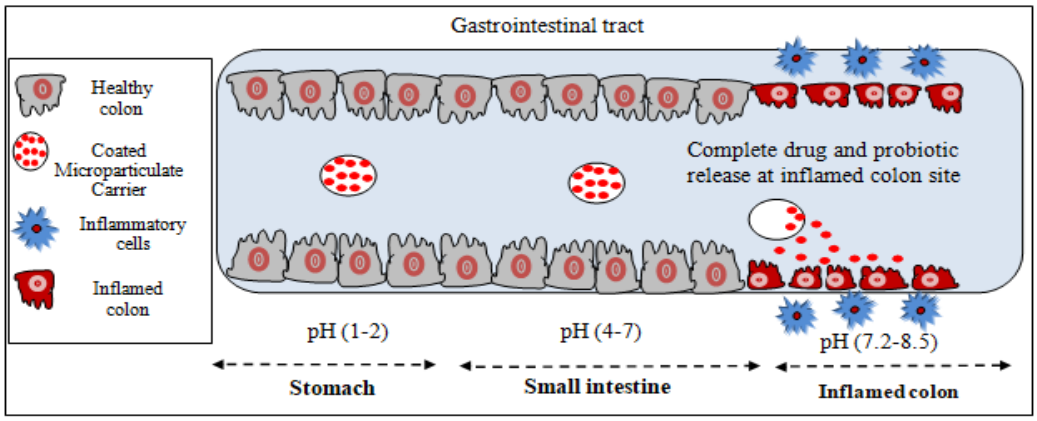


Figure: 1.7 Diagrammatic representation showing research envisaged.

1.16 RESEARCH AIM AND OBJECTIVES

1.16.1 Research aim

The current study aims to design, develop and evaluate novel drug delivery systems (microparticles and pellets) containing Mesalamine and probiotic strain at the colon site to ensure optimum anti-inflammatory and achieve increased therapeutic effectiveness and the least extent of side effects.

1.16.2 Research objective

To achieve the aim of this research, the following objectives were proposed:

1. To develop microparticulate formulations of Mesalamine with probiotics.
2. To optimize critical variables (materials) for the development of microparticulate formulations.
3. To screen and select the best strain of probiotics based on antioxidant and anti-inflammatory properties.
4. To carry out accelerated studies of optimized formulations.

5. To evaluate *in vivo* efficacy and pharmacokinetics of developed formulations.

1.17 PLAN OF WORK

1. **Extensive Literature Survey**
2. **Preformulation studies**
   - Drug morphology
   - Physical state
   - Melting point
   - Partition coefficient
   - Solubility study
   - FTIR spectroscopy for compatibility study
3. **Analytical method development and validation**
   - UV spectrophotometric method
   - HPLC Method for drug stability assay
4. **Screening of probiotic strain based on the antioxidant and inflammatory property**
   - Cell line study (Caco-2 cell line) to determine the anti-inflammatory efficacy of probiotic
5. **Preparation and optimization of material and process variables during formulation**
   - **Method used for the preparation of microparticles**
     - Preparation of microparticles by dehydration technique
     - For coating of microparticles oil-in-oil solvent evaporation method
   - **Method used for the preparation of pellets**
     - Pellets prepared by extrusion and spheronization method
     - Coating of the pellets using accela-cota instrument
   - **Microparticles formulation**

- ❖ Independent variables (material): Drug: polymer, Surfactant concentration, Stirring speed, the concentration of cross-linker
- ❖ Dependent (Response variables): Particle size, Entrapment efficiency, *in vitro* drug release

➢ **Pellets formulation**

- ❖ Independent variable(material): Drug: polymer, Binder, Bulking agent, the rotation speed of spheronizer and extruder (RPM), Rotation time
- ❖ Dependent (Response variables): Particle size, Entrapment efficiency, *in vitro* drug release

6. *In vitro* characterization of optimized formulation of the microparticles

- ➢ Drug loading efficiency
- ➢ Drug entrapment efficiency
- ➢ Particle morphology (SEM)
- ➢ Particle size analysis, polydispersity index, Zeta potential
- ➢ *In vitro* drug and probiotic release
- ➢ FTIR spectroscopy
- ➢ XRD
- ➢ DSC
- ➢ Storage stability studies

7. *In vitro* characterization of optimized formulation of pellets

- ➢ Drug loading efficiency
- ➢ Drug entrapment efficiency
- ➢ Particle morphology
- ➢ Sphericity studies
- ➢ Micrometric properties (Angle of repose, Bulk density, Tapped density, Hausner's ratio, Carr's index and friability.
- ➢ Mucoadhesive study
- ➢ FTIR spectroscopy

- XRD
- DSC
- *In vitro* drug and probiotic release
- Storage stability studies

8. *In vivo* studies

- *In vivo* efficacy studies using TNBS –induced colitis model in Wistar rat
- Morphological analysis
- Biochemical studies
  - Determination of Myeloperoxidase (MPO) colonic activity
  - Determination of Lipid peroxidation(LPO) colonic activity
  - Determination of Glutathione(GSH) colonic activity
- Measurement of Serum-Markers
  - Determination of C-reactive protein
  - Determination of Erythrocyte sedimentation rate (ESR)
  - Determination of White blood cell count
- Histopathological studies

9. Pharmacokinetic studies for both formulations (Microparticles and pellets)

10. Statistical analysis

11. Thesis Compilation, the publication of research outcomes, and thesis submission