Inflammatory Bowel Disease

Chapter 6

Advanced Drug Delivery Systems for Inflammatory Bowel Disease (IBD)

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1. Introduction

1.1. Targeted drug delivery system

Definition

Targeted release describes drug release directed towards isolating are concentrating a drug in a body region, tissue or site for an absorption or for drug action [1].

Oral Targeted drug delivery

Oral route is the most popular route for administration of drugs meant for systemic effects. The oral route is very extensive and different parts of it have different structure and different physic chemical characteristics. These characteristics can be utilized for the targeting of drugs to the different parts of the gastrointestinal tract. If the drug is targeted to colon, the formulations have to be designed accordingly [2].

Types of Targeted oral formulations

1. Drug delivery systems targeted to stomach/duodenum.
2. Drug delivery systems targeted to the small intestine
3. Drug delivery systems targeted to the colon.
4. Drug delivery systems targeted to Lymphatics

1.1.1. Targeted delivery systems for stomach /duodenum

There are several methods targeting the dosage from to stomach and duodenum. The few principles of those have discussed below. The density of the dosage forms is adjusted to lesser than the density of the gastric fluid in order to make the dosage form float on the gastric fluid. Effervescent type of Tablets are also being used in this category by utilizing their buoyancy characteristics. Targeting may also be achieved by creating large size dosage forms because of their larger size their transit time is increased and they can reside in the stomach for longer time. But the main disadvantage of this method is it is very difficult to swallow such large dosage forms. Now a day the most useful technique is the usage of bioadhesive materials and it can adhere to gastric epithelium or to mucin. Layer though many such substances are not very effective in the gastric pH. Stomach targeted systems are useful in targeting vitamins, minerals, antibiotics, antinausents, hypotensives and sedatives.

1.1.2 Targeted drug delivery systems to the small intestine

Enteric coated products are the finest examples of small intestine targeted delivery. Enteric coating is obtained by using different types of enteric coating materials. Polymers selection plays critical in achieving targets to the lower part of the small intestine.

1.1.3 Targeted delivery system to lymphatic systems

Lymphatic consist of a network of vessels throughout large and small intestines. Drugs targeted to them avoid hepatic first pass metabolism. The large molecular drugs are effectively absorbed through lymphatic and so are antigens, hydrophobic drugs and like streptomycin and gentamycin which are hydrophilic or insoluble.

1.1.4 Targeted drug delivery systems to the colon

As the colon represents the most distal segment of the gastrointestinal tract, targeting this region of the gut can be problematic. Although the rectal route can be used to gain access to the colon via the administration of suppositories and enemas. Such formulations rarely succeed in spreading beyond the descending colon, with little or no drug reaching the proximal colon [3]. Also, the rectal route is not convenient or acceptable for most patients. The oral route is therefore the preferred mode of administration [4].

Orally administered drugs are generally administered in the form of immediate release or modified release formulation. While the former are intended to release the drug in the stomach, so providing rapid absorption, the modified release system is designed to extend or delay the release of the drug in the gastrointestinal tract. Modified release systems are further subdivided into a single unit or multiple unit dosage forms such as Tablets and capsules, usually move through the gut intact, whereas multiple unit dosage forms, such as pellets, granules or mini

–Tablets, exist as discrete entities in the gastrointestinal tract. traditionally, single unit systems have proven to be more popular than multiple unit preparations because of their ease and cost of manufacture. However, the fundamental distinction in design between the two systems gives rise to their differences in in-vivo behavior. The small size and divided nature of multiple unit systems permits more uniform gastro intestinal transit particularly gastric emptying and colonic transit and drug release characteristic than with single unit systems. The chances of dose dumping, and conversely incomplete drug release, are also less likely with multiple unit dosage forms, Specific details of the gastro intestinal handling of the two types of system can be found elsewhere [5]. On balance, the multiple units provide the more reliable platform on which to base development of oral colonic release drug delivery systems. Colon specific drug delivery via the oral route is simple concept. The formulation must retard the drug release in the stomach and small intestine but allow the drug release in the colon. However, this is difficult to achieve in practice because the formulation will be exposed to a range of condition and environments on passage down the gut, including pH, enzymes electrolytes, transit time and pressure. More over these parameters are subject to considerable inter and intra individual variation and are also affected by the disease, which makes colonic delivery via the oral route a challenging proposition [6,7]. In the context of the colonic targeting, the exploitable gastrointestinal features have include pH, transit time, pressure and micro flora. On the basis of these features, range of approaches has been proposed and system developed, but the majority of these have not ever progressed beyond the bench, with very few reaching the stage of clinical evaluation.

2. Drug Deliveries to Colonic Region

Colon specific drug delivery is very much appreciated in treating colonic disorders, such as Crohn's disease, ulcerative colitis and irritable bowel syndrome. There is an increasing number of studies performed in colon specific drug delivery not only in treating the colonic diseases but also in utilizing the positive characteristics of colon especially the physiological and environmental advantages of the colon in developing controlled and other drug delivery devices. Colon is targeted not only for the local action in the colon but also for the systemic bioavailability. This is successfully proved in drug delivery of peptides and proteins through the colon. The drugs that are unsTable in the upper gastrointestinal tract can be successfully exploited as colon delivery. In such cases, the enzymatic degradation and hydrolysis of peptide drugs are prevented that assures the systemic availability of the drugs, the poorly absorbed drugs which undergo first pass metabolism and drugs used in the treatment or arthritis and asthma are the drugs that can be given as colonic drug delivery.

Several approaches have been explored in Colon-Specific Drug Delivery System (CSDDS) to treat the colonic diseases by targeting and delivering the drugs to the colon and is an alternative method to overcome the barriers of successful therapy. Topical [Inflammatory

Bowel Disease (IBD)], local (pancreatitis) and the systemic availability of insulin are possible with the colonic drug delivery system. The prime objective of the drug delivery is to increase the pharmacological activity, reduce side effects and prevent drug from degradation. Since CSDDS is a safe and an advanced technique, it offers all the above three advantages. The drug delivery to the colon has to cross several physiological barriers like degradation of the drug in the gastrointestinal tract (GIT) and absorption of the drug in the GIT before it reaches the colon. The diseases that affect the GIT may affect the drug delivery in the colon. Although, several approaches for drug delivery in the colon are available most of them are based on the following fundamental mechanisms such as biotechnologically developed DDS, pH sensitive polymeric systems, microflora activated drug release, time dependent DDS, and prodrugs.

For evaluation of CSDDS, several methods of alternative techniques are available such as a modulator fermentor method, simulated human microbial eco- system method, and system using rotating beads to mimic the motility and hydrodynamics of the colon. The result obtained from each method differs according to the design of the methods used for evaluation. The conventional United States Pharmacopoeia (USP) dissolution testing using different buffers is simple, convenient and efficient in finding release profiles of the drug formulations in CSDDS.

2.1 Advantages

- SuiTable for drugs degraded by the enzymes in the stomach and small intestine.
- Provides enhanced absorption of poorly soluble drugs by offering long retention time in the colon.
- Wastage of drugs by unnecessary systemic absorption is reduced and intact form of the drug is saved till it reaches the target site.
- If the area to be treated is a colon, the absorption and delivery of the drugs in other regions of the GIT is not necessary, especially for drugs harmful to those regions.
- Increases the overall reliability of the therapy.

2.2 Disadvantages

- Substantial variation in gastric retention time may affect drug delivery.
- Diseased condition may affect the colonic transit time and the drug release profile, e.g. diarrhea, ulcerative colitis and IBD.
- pH levels of the colon may vary between individuals due to diseases, chemotherapeutic agents used, state and temperature of food consumed.

• Colonic microflora may be affected by diet and diseases that affect the enzymatic action of colonic area, which leads to poor in *vitro-In vivo* correlation, hence necessary to carry out the bioavailability study.

2.3 Polymers used in colonic delivery

Eudragit-L100, Eudragit-S100, Eudragit- L30D, Eudragit-FS30D, Eudragit-L55, Polyvinyl acetatephthalate, HPMCP, HPMCP-50, HPMCP-55, Cellulose acetate trimiliate, and Cellulose phthalate are the polymers commonly used and their thresholdpH falls between 4.5-7.0, which is suiTable to explore it as colonic drug delivery device.

2.4. Techniques used in colonic deliveries

2.4.1. Timed-release systems

The mechanism of timed-release CSDDS is that it can resist the acidic environment of the stomach and passes the stomach intact and reaches the distal part of the small intestine where the drug is released after pre- determined lag time. A good example is using Diltiazem hydrochloride as a model drug for Enteric coated-Timed release-Press coated (ETP) Tablet. In the ETP Tablet, the outer shell is coated with Hydroxy Propyl Cellulose (HPC). The *in vitro* study on an ETP Tablet in pH 1.2 and pH 6.8 fluids show the timed release as well as acid resistance characteristics [8]. For colon targeting, the formulation itself should suppress the drug release completely for 2 hours in the stomach and should release the drug rapidly after 3±1 hour, which is considered as lag time. Enteric-coated capsules with HPC drug container are also available for release of drug specifically in the colon. In this, the capsules are made of Ethyl Cellulose (EC) and drug container with the low substituted Hydroxy Propyl Methyl Cellulose (HPMC). Water penetrates into the drug container through the micropores of the capsule shell that influence EC and the whole system to release the drug by disintegration [9].

2.4.2. Pressure controlled devices

The drug, 5-amino salicylic acid (ASA), is prepared by pressure controlled DDS after administration, both in the beagle dogs and human volunteers5-ASA appears in the blood plasma after 3 to 5 hours known to be the time taken for the system to reach the colon [10]. The pressure controlled colon delivery capsules for liquid preparation, time controlled colon delivery capsules for liquid and solid preparation, and Eudragit-S coated Tablets for solid preparation are the three kinds of DDS in pressure controlled devices. The drug release from the solid preparation was better and well controlled as compared to that of the other two preparations [11].

2.4.3 Biodegradable nanoparticles

The study was conducted in Wistar rats with the drug, Rolipram, an anti-inflammatory model drug. Rolipram was prepared as a nanoparticle with poly (lactic-coglycolic acid) given once orally for four consecutive days. Previously, male Wistar rats were induced forexperimental colitis by 2,4,6- Tri Nitro Benzenesulphonic acid (TNBS) to assess the activity of Rolipram on induced colitis. All nanoparticle formulations proved to be as efficient as the drug in solution in mitigating the experimental colitis. The administration of Rolipram nanoparticle and solution significantly decreased the clinical activity score and myeloperoxidase activity. But during the next 5 days, when animals Were kept without the drug treatment, the solution form of Rolipram showed significant relapse but the nanoparticle slowed the continued inflammation levels. When compared to the solution, Rolipram nanoparticle showed a reduced adverse effect index. This was because Rolipram nanoparticle had the potential to retain the drug from systemic absorption. Regular treatment envisages the absorption in the small intestine that causes significant side effects [6]. The biodegradable nanoparticle drug delivery systems plays a successful role in the treatment of IBD when compared with existing drug delivery systems.

2.4.4 DDS targeting immune regulating cells

A study on oral DDS targeting immune regulating cells ameliorates mucosal injury in TNBS-induced colitis is an ideal example for delivery system, which targets the immune regulating cells. The study focused on the immune regulating cells in thecolonic mucosa, which is important inpatients to examine the therapeutic effect of Dexamethasone microspheres over Dexamethasone alone, the microspheres were given to rats with TNBS induced colitis. A macroscopic score, histological score and myeloperoxidase activity were assessed. All the above three scores treated with Dexamethasone microspheres were significantly lower than those treated with Dexamethasone alone.

The gene expression of pro-inflammatory cytokines and Cyclo-oxygenase-2 in rats treated with ‘Dexamethasone microspheres was down-regulated, compared with that in rats treated with Dexamethasone alone. The number of PCNA (Proliferating Cell Nuclear Antigen) -positive cells in the Dexamethasone microsphere group were larger than in the group treated with Dexa¬methasone alone. In addition, Dexamethasone microspheres suppressed NF-KB activation in TNBS induced colitis. Therefore, the overall report stated that the oral administration of Dexamethasone microspheres appears to be more advantageous than Dexamethasone alone in treating the mucosal injury in TNBS induced colitis. Thus, the CSDDS could be an ideal therapy for human IBD [7].

2.4.5 Gene delivery to inflamed colon

The usage of recombinant adenovirus for the gene delivery into epithelial and sub-epithelial cells of the inflamed colon are useful in treating colonic inflammation [12]. GIT is a potentially attractive target for gene delivery. Local administration of recombinant adenoviruses with the normal AdCMVβGal (a vector used as a reporter gene) and an adenovirus with the modified fiber structure [Adz. F (pk7)] could facilitate the most successful gene delivery when the colon is targeted. The signal transduction proteins with suiTable therapeutic potential can be delivered in the colonic tissue by this method of gene delivery. The local administration of adenoviruses into an experimentally induced colitis by hapten reagent (TNBS) was better treated by local administration rather than other routes of administration. Finally, AdCMVβGal adenoviruses with modified fiber structure produced 10 to 40 folds higher reported gene activity in spleen T-cells and lamina propria mononuclear cells of colitis mice compared with standard AdCMVβGal vectors.

2.4.6 MAb targeted therapy for colonic cancer

Monoclonal antibodies (MAbs) help the immune system to respond quickly to infection by increasing the sensitivity to recognize the infection when it occurs again. For colon targeting, MAbs can be prepared in a laboratory and utilized in treating colonic cancers. MAbs act by identifying and locking the proteins of the cancer cells and triggers the body's immune mechanism to attack the cancer cell in order to kill the cells. MAbs can be attached either with a cancer drug or with a radioactive substance and deliver them directly into the cancer cell [13]. Epidermal growth factor receptor (EGFR) is the receptor that has been located on the surface of the cancer cells. The MAbs like cetuximab and bevacizumab locks EGFR and may prevent the cancer cells to grow or divide. Cetuximab is given as an infusion through the vein to treat advanced metastatic cancer in combination with Irinotecan and 5-fluorouracil.

2.4.7 Bio-adhesive polymers

Bio adhesion is a process by which the drug moiety remains in contact with a particular organ for an extended period of time. The polymers like polycarbophils, poly-urethane, polyethylene oxide, polypropylene oxide and copolymers are considered as suitable material for bioadhesive DDS. The two major factors that affect the colonic drug availability are the dissolution of the dosage form before it reaches the colonic environment and the absorption of the drug in the upper part of the GIT or other than colonic region. This may be the reason behind the side effects and low colonic concentration of the drug. The water soluble polymer of [N-(2-hydroxy propyl) meth-acrylamide] (HPMA) co-polymer was studied by Kopececk.14 This DDS was designed with both bio-adhesion and site specific drug delivery of 5-ASA with HPMA co-polymer that contains saccharide units complementary to mucosal lectins of the GIT as carriers.

2.4.8 Hydrogels

Hydrogels are the potential carriers for drugs as well as other candidates to be targeted to the colon. The types of hydrogels can be classified as azo-aromatic hydrogels, inulin hydrogels and dextran hydrogels. Dextran hydrogen is further classified into methylated dextran hydrogels and activated dextran hydrogels [15,16] In hydrogels, the acidic co-monomers are the gel which remains intact in the stomach and liberates the drug after it reaches the colon. This is due to the cleavage of the crosslink between the drug and the hydrogel [17] Azo-aromatic hydrogels showed faster *in vitro* release of 5-fluorouracil in presence of azo reductase in the culture of the intestinal flora [18]. Methylated dextran hydrogels and activated dextran hydrogels show better degradation than the inulin hydrogels as the dextran degradation is influenced by dextranase [19]. The degradation process was studied in the human colonic fermentation model [20]. Inulin hydrogels and dextran hydrogels serve better delivery of the drugs in the colon. The *in vitro* and in vivo degradability of the hydrogel depend on the swelling property of the hydrogels and cross links seen in the hydrogels also play a vital role in the release of the drug. The low cross-linking density hydrogels can degrade faster than the hydrogels with high cross-linking density. There is an enhanced release of the bovine serum albumin from the hydrogels by the addition of the dextranase in the dissolution medium [21,22].

1.4.9 pH regulated pellets

The anionic polymer like Eudragit L and Eudragit Sare used for the preparation ofCS-DDS which isimpermeable to water at low pH. The techniques like extrusion, spheronization and pelletization are used to develop pH sensitive matrix pellets for colon targeted drug delivery [23].The studyas focused to assess the role of the organic acid in the enteric matrix granules. The result observed that there was retardation in the *in vitro* release of the model drug but the corresponding effects was not seen with thein *vitro -in vivo* study [24].

2.4.8 Biodegradable polysaccharides

There are several poly- saccharides that have been used successfully in CSDDS. Polysaccharides such as amylose, Guar gum, Pectin, Chitosan, inulin, cyclodextrin, chondroitin sulphate and dextrans are some of the examples used in the CSDDS [25, 26] Their resistance to the digestive enzyme and the biodegradable property in the colon are the characteristic features, which develops polysaccharides as CSDD polymers [27].

2.4.9 Osmotically regulated systems

These systems are operated by the osmotic changes in the inside and outside of DDS. Around 5 to 6 push-pull units are seen in the osmotically regulated system [28]. Each unit is 4mm in diameter and perfectly placed in the hard gelatin housing or encapsulation. The

push-pull system, Oros-CT (Alza Corporation, California, USA), contains a drug layer and the osmotically sensitive layer. After swallowing, the hard gelatin cover is dissolved but DDS remains intact in the upper GIT because of the enteric coating given in the system. As soon as the unit enters into the intestine, the water intake of the osmotically sensitive layer is initiated, which initiates the drug release from DDS that is slow-flowed into the intestinal environment through the orifice which is previously drilled in the system. To treat ulcerative colitis, each system is designed to deliver the drug after 3 to 4 hours to prevent drug delivery in the small intestine. The advantage of the system is that the drug release can be maintained constantly for 4-24 hours.

2.4.10 Prodrugs

Prodrugs are classified as also conjugates, glycoside conjugates, cyclodextrin conjugates, dextran conju¬gates and amino acid conjugates [29,30] In this approach, active drug is cleaved from the carrier molecule via the action of enzymes derived from the colonic microflora and redox potential of the colon. The finest example is sulphasalazine, which is chemically salicyl-azo sulphapyridine, in which salicylate radical and sulphapyridine are linked by an azo-bond. When ingested, the bulk of the sulphasalazine reaches the colon intact, where the azo-bond is cleaved by colonic bacteria and the liberation of sulphapyridine and 5-ASA occurs [31].

3. Dissolution Studies of Colon Drug Delivery Systems

In Multi Ph Media Using Usp Apparatus II AND III

The evaluation of colonic delivery systems can be carried out to study the release profile in buffer solution of pH 1.2 that resembles the artificial gastric fluid and pH 6.8 for intestinal fluid and pH 7.2 to mimic the pH conditions of the distal part of the intestine and colon. USP dissolution apparatus II (paddle) and apparatus III (reciprocating cylinder) coupled with automatic sampling devices [32] and software was used to develop a testing procedure for acquiring release profiles of CSDDS drug formulations in multi-pH media. In a study, acetaminophen was used as a model drug prepared as CSDDS and used for the study of release pattern for CODES™ (Yamanouchi Pharma Technologies, Japan). Apparatus III has been demonstrated to be more efficient than that of apparatus II by offering flexibility in sampling time, agitation rates and medium changes. This concludes that apparatus III showed high efficiency in the study of *in vitro* evaluation of CSDD devices. Also pH 5.0 medium shows rapid drug release than other pH mediums, but dipping length and paddle speed also plays a significant role in drug release.

In Vitro Drug Release Studies

This test is to find out the intactness of coatings and carriers in simulated conditions of the stomach and intestine. To assess the drug release in the stomach, 0.1N Hcl is used as dissolution medium and studied for 2 hours (mean gastric time). After that, the drug release profile has been studied in phosphate buffer for 3 hours (mean small intestine transit time) to assess the intactness in the small intestine. From this, the Samples are collected at different time intervals and determined for drug release.

In Vitro Enzymatic Degradation Test

This test can be carried out by two methods: In the first method, enzymes like Pectinase and dextranase of the rat, guinea pig or rabbit's cecal content may be added in the buffer medium. The samples are collected at frequent intervals and studied for drug release profile. In another method, carrier drug system is incubated in the fermentor and suiTable buffer medium is selected which contains colonic bacteria like Streptococcus faecium or B. ovatus. The amount of drug release at a particular time is directly proportional to the rate of degradation of polymer carrier. Generally, it is very difficult to design an ideal *in vitro* model for evaluation of colonic DDS. The conditions like pH, volume, rotations per minute, bacteria and their enzymes, and enzyme activities are affected by food, disease conditions and physical stress that make the design of ideal *in vitro* model for evaluation extremely critical.

3. Recent Therapeutic Approaches in IBD

Inflammatory bowel disease (IBD) is an idiopathic disease. Novel drug delivery systems are very successful in the treatment of colonic diseases. The colonic environment can be explored for local and systemic delivery of drugs and other bioactive compounds such as hormones, insulin, vasopressin and other plant ingredients. The colon is the major region of the GIT. The common colonic disease such as Diverticular Inflammatory Bowel Disease (IBD) which includes Crohn's disease and ulcerative colitis, colitis ulcerosa diversional colitis, ischemic colitis, colon cancer and lymphoma of the colon can be treated successfully by modern therapeutic approaches. In past decades the general pathophysiology of inflammatory bowel disease was described on the basis of clinical manifestation. The investigators and clinicians are struggled to provide the effective therapy for IBD due to its dismaying clinical manifestation The causes of inflammatory bowel disease is multi factorial and may be resulted from inappropriate activation of mucosal immune system, inflammatory responses, generic factors, candidate genes, chromosome location etc., The infectious organism such as Escherichia coli, measles virus, cytomegalo virus and factor like saturated fats, milkproducts, allergic foods may also be the cause of the IBD. General pathophysiology of ulcerative colitis and Crohn's disease is limited to large intestine often inflammation and ulcers occurs in the inner lining of

the large intestine or in mucosal layer. The ulcerative colitis resulting in diarrhea, blood and pus. Crohn's disease otherwise called regional enteritis. Crohn's disease involves any part of the gastrointestinal tract from mouth to anus with the inflammation extending through the bowel wall to the serosal surface. Both the diseases used to have waxing and waning intensity and severity [33,34,35].

Crohn's disease and ulcerative colitis significantly differ from each. The treatment methods are common to both the diseases. Many extra intestinal manifestations are shared by both the diseases occurs in adults and children. The signs and symptoms of ulcerative colitis involves diarrhea with the presence of blood and mucus. Weight loss, abdominal pain, painful bowel movements, abdominal cramps and extra intestinal symptoms like arthritic knees may be observed in youngsters. The pathophysiology of ulcerative colitis showed an increased amount of colonic sulfate reducing bacteria. This may be due to the result of higher concentration of hydrogen sulfide toxic gas. Some reports suggested that sulfur containing red meat, alcohol consumption also increased the disease relapsing in patients in remission. The ulcerative colitis occur in 38-100 for every 100,000 in the US. The disease occurs predominantly in northern countries0.1%population. Ulcerative colitis has no known cause and it is treated as autoimmune disease.

4.1 Management of IBD

The treatment approaches depending on the severity of the disease. Pharmacotherapyis significantly successful in the treatment of ulcerative colitis. The optimized medical approach differs with a physician. The main objective of the treatment is to induce the remission followed by the maintenance therapy to prevent relapse. The drug treatment involves aminosalicylates such as sulfasalsasine corticosteroids (Prednisolone), immune suppressive agents (azathioprine) and biological agents such as infliximab. Ulcerative colitis generally be cured by surgical removal of the large intestine which is not recommended in the early stages. Drug delivery systems with modified and targeted drug delivery to the site and prodrugs approach can also successfully used in IBD management. The list of the drugs approved commonlyfor ulcerative colitis and Crohn's are given below.36 [37].

Corticosteroids: hydrocortisone, methylprednisolone, Prednisolone as I.V route, oral or retention enemas.

5-Aminosalicylic Acid Compounds

Sulphasalazine combination of sulphapyridine and 5-aminosalicylic acid joined by an azo - bond. Poorly absorbed, split by bacteria in the colon. 5-ASA part is the active moiety; sulphapyridine part can cause sulfonamide toxicity. Reduces relapses in UC and used for treatment of exacerbations.

Side effects – headache, nausea, vomiting, anorexia – commoner in slow acetylators (of the sulfonamide part). Lupus-like syndrome. Rash, fever, lymphadenitis, agranulocytosis. Reversible male infertility.

Mesalamine - 5 - ASA. Delayed release, pH- dependent preparations to allow release in the colon. Better tolerated than sulphasalazine, but still carries risk of hematological side effects.

Olsalazine- 2 molecules of 5-ASA linked by azo-bond split by colon bacteria.

Balsalazide prodrug- of 5-ASA

Immunosuppressants- Azathioprine

Metronidazole- Perianal Crohn's disease.

Infliximab- severe active Crohn's disease refractory to treatment with steroids.

Monoclonal Antibody- Inhibits the pro-inflammatory cytokine TNF-α.

NSAID- Ibuprofen, Diclofenac, Indomethacin Analgesic – rapid (full effect within 1 week) Anti-inflammatory (full effect within 3 weeks)

4.2 Various studies on colonic pH conditions

The various studies conducted worldwide by following different techniques registered the pH changes in normal and IBD colon. The Table 1.1 displays the colonic pH conditions in the normal healthy volunteer patient with ulcerative colitis and Crohn's disease studied by various reserarchers. Changes in the intestine and colonic pH also considered as an important factor in the treatment of ulcerative colitis and IBD. The design and development of novel treatment and novel drug delivery systems are also significantly influenced by the changes in the luminal pH conditions and that should be considered in the treatment of IBD. The formulation also developed to deliver an active agent directly to the inflammation site. This approach reduces the absorption of drugs in the upper GI tract as well as the systemic side effects. This method involves pH dependent drug delivery systems (Asacol, Mesacol and Salofalk). Another common technique involves bacterial enzymatic metabolism, (sulphasalazine, olsalazine and balsalazide) which also affected by changes in colonic pH.

Table 1: Ph Conditions in Normal and Inflamed Colon

Sl.no	Study	Patient	Smallbowel pH		Colonic pH	
			Proximal	Distal	Ceacum/ right colon	Left colon
1	Fallingborg 199938	39 normals	6.4	7.3	5.7	6.6
2	Fallingborg 199839	13 normals	6.4	7.4	5.8	-
3	Raimundo 199240	7 normals	6.6	7.4	6.7	-
4	Watson 1972[41]	2normal + 7 GI disorder cases	5.5-7.5	6.5 -7.5	5.5-7.5	6.5-7.5
5	Evans 198842	66 normals	6.6	7.5	6.4	7.1
Patient with Ulcerative Colitis						
6	Raimundo 1991[43]	7 acitive 6 inactive	6.1 5.9-6.6	7.2 6.9-7.4	4.7 4.9-5.5	- -
7	Fallingborg 1993[44]	3 active 3 very active	Normal Normal	Normal Normal	Normal 2.3-3.4	- -
8	Nugent 2000[45]	6 active	7.3x`	8.3	6.7	6.7
9	Press 199846	7 active 4 nactive	6.8 6.6	8.2 7.9	7.2 6.5	6.8 6.5
10	Eve 199947	4 active	6.5	6.8	5.5	7.5
Patient with Crohn's disease						
11	Fallingborg 1998[39]	9 withileo ce-cal resection	6.3	7.3	6.7	NA
12	Sasaki 1997[48]	3 acitive 1 inactive	7.2	7.8	5.3	5.3
13	Eve 1999[47]	12 active	6.5	7.5	6.2	6.5
14	Press 1998[46]	5 active 7 inactive	6.5 6.8	7.9 8.2	6.5 6.5	6.5 6.5

NA- Data not available

4.3 Treatment methods for IBD and its limitations

Treatment usually based on reports of clinical history, physical parameters endoscopy, radiology, histology and regular laboratory tests. The study on these reports gives the clear idea about IBD and also distinguishes the ulcerative colitis and Crohn's disease but it is very difficult to distinguish ulcerative colitis and Crohn's disease in at least 10% of the population for them IBD is limited to the colon. [49,50].

Long time management of inflammatory bowel disease involves the drug therapy and lifestyle management. The therapy may be started with anti diarrheal in the beginning to give the symptomatic relief and the treatment should be focused on reducing the inflammation once the symptoms are subsiding. A correct diet and nutrition's are advisable as a supporting measures for the successful IBD management. A drugs such as 5 amino salicylic acid (Mesalamine) were used in the treatment of IBD [51]. 5–ASA widely replaces the sulfasalazine for its safety and less adverse effects. Mesalamine (5-ASA) not considered as the very potent anti-inflammatory agent but shown to be effective in IBD patients but also fails to show significant improvement in the set of patients Affected with IBD.

4.3.1 Important considerations of amino salicylates.

- Amino salicylates are unsTable in gastric acid
- Rapidly absorbed in the small intestine.
- The safety and tolerability of amino salicylates are the reason behind the withdrawal of these agents.
- The greater number of withdrawals were reported with sufasalazine 3g/day than balsalazide 6.75g/day [52,53].
- Balsalazide has a more adverse drug reactions than delayed release Mesalamine [54].
- The unsTable nature of Mesalamine in the upper GI region paves the way for developing them into novel drug delivery systems such as delayed release formulation based on enteric coating pH dependent release system which breaks at ileal or colonic PH or environment. Prodrug based systems, microflora activated system based on poly sachharides such as Pectin, Guar gum, Chitosan, timed release system, etc. [55, 56].

4.3.2 Corticosteroids

- Corticosteroids are recommended when 5–ASA compounds are inadequate in producing the expected results.
- Topical corticosteroids (enemas) used in the patient with ulcerative colitis, Prednisolone 60mg/day is used orally in the treatment of ulcerative colitis and Crohn's disease. The mechanism of action of corticosteroids was well known and is acts by inhibiting the several inflammatory pathways and stimulation of lymphocyte apotosis [57].
- Corticosteroids are known for its systemic side effects in which adrenal suppression and osteoporosis,corticosteroids induced hypertension and diabetes are well noted.

• Intravenous administration of prednisolone also recommended when the condition of the patient is severe [58,59].

• The budenonide usage may minimized the side effects of prednisolone and also available in the form of enema [58,59].

• Budensonide efficacysome what less than the conventional corticosteroids due to first pass metabolism. Fluticasone was not effective in distal disease [60,61].

• The main disadvantage is usage of these corticosteroids are difficult in crohn's disease than ulcerative colitis due to the variation in colonic pH, transit time and bacterial metabolism[63].

4.3.3 Immuno modulatory therapy

• Azathiopurine and Mercaptopurine (6-mercapropurine) are the most commonly used immune modulator. These are the derived products of thioguanides. This recommended when corticosteroids can not be withdrawn from the patient.

• Although azathiopurine earlier reported for side effects and producing increased risk of lymphoma which is became a highly recommended immuno suppressive agent [64,65,66,67].

• Efficacy of the drug was mostly depend on the dose, and the optimal dosage was 2.0-2.5 mg/kg/day and 1.0-1.5mg/kg/day was found optimal for Azathiopurine and Mercaptropurine respectively [68].

• The side effects of these agents are limit the usage of Azathiopurine and Mercaptopurine. So that Azathiopurine should be prescribed with caution and step wise approach. The serious adverse effects includes bone marrow suppression, variation in the white cell counts [69,70].

• Cyclosporine is recommended in the patient with steroidrefractory ulcerative colitis [71]. Cyclosporin inhibits the cellular immune response by blocking cytokinine production by T lymphocytes through calcineuron dependent pathway [72,73].

• Cyclosporins provide the rapid onset of action by intravenous route with significant clinical improvement about a week [74]. cyclosporins should not be recommended in the treatment of crohns disease which may cause severe perianal or cutaneous fistula [74].Although cyclosporins are considered as a alternative for corticosteroid therapy their adverse effects limits the usage.

• Tacrolimus and mycopholate mefetilalso effective in the treatment of IBD. Tacrolimus is a macrolide immune suppresent which inhibits the immune response through a calcineurin

dependent pathway [75]. Tacrolimus can induce remission in adults and children [76,77]. Recent days studies supported the usage in the treatment of corticosteroids dependent crohn's disease for remission and relapsing [78,79,80,81].

- It can be given as weekly injection 15mg and 25 mg weekly by IM and SC respectively. The mechanism of action of this drug is unclear and also known for it's side effects such as immune suppression, interstitial pneumonitis associated with non productive cough, dypnea and hepatic fibrosis.

4.3.4 Biological agents

- Biological agents such as infliximab (protypical anti – TNF agent) made the significant advancements in the treatment of crohns disease. The mechanism of action of infliximab yet to be explained completely. It is a chimeric monoclonal antibody. It is an agent which shows the significant results in the treatment of crohn's disease but not in ulcerative colitis [82,83].

- Basiliximab was showed significant clinical remission in ulcerative colitis in pilot scale study [84].

- Daclizumab infusion showed the decreased clinical activity score in refractive ulcerative colitis patients. Natalizumab demonstrated a significant clinical response in active ulcerative colitis patients [85]. The rapid immuno modulatory agents are known for its severe adverse effects which sometimes fatal.

4.3.5 Probiotics and antibiotics in IBD

The studies worldwide supported the recognition of antibiotics in the treatment of IBD [86]. Other reports on probiotic also revealed that probiotic can be used as a supporting agent in the treatment of IBD. The usage of antibiotics in the treatment of ulcerative colitis is limited. In crohn's disease metronidazole (750mg/day/tid) found to be effective. The side effects of metronidazole such as neurotoxicity to be taken into consideration before and during the treatment. Metronidazole is effective in the treatment of crohn's disease but showed no significant respose in the treatment of ulcerative colitis.

The administration of probiotics is an excellent supporting approach in the management of crohn's disease which is free of any side effects [87,88]. Lactobacillus acidophilus LA1 have the reported effect in immune enhancement adherent to human intestinal cells and balancing the microflora. Lactobacillus acidophilus NCFB is effective is effective in lowering faecal enzyme activity decreased faecal mutagenecity in the treatment of rotavirus diarrhea crohn's disease and antagonistic against carcinogenic bacteria. Lactobacillus casei shirota is effective in prevention of intestinal disturbances balancing intestinal bacteria and immune enhancement. Bifido bacterium bifidum is effecvtive in treatment of rotavirus diarrhea and

balancing intestinal microflora. Lactobacillus gasseric (ADH) is effective in the treatment of IBD [89,90,91,92].

4.3.6 Importance of onset of action in IBD treatment

• Pharmacotherapy brought the excellent changes in the management of ulcerative colitis and crohn's disease. The onset of action plays a major role in various treatment. In case of the IBD onset of action is most important in management, maintenance of remission of disease.

• In multicentre trial revealed that onset of action of balsalazide was earlier than Mesalamine [93].

• The patient with Sulfasalazine intolerance can be successfully treated with Balsalazide, Olsalazine or Mesalamine.

• Although corticosteroids have role in maintenance and remission of ulcerative colitis. Their efficacy relying on rapid onset of action and anti inflammatory activity which gives the consistency in treatment with corticosteroids in ulcerative colitis.

• The study on immunomodulatory agents revealed that the clinical benefits of thioguanine derivatives desired only after 4 months of therapy in Crohn's disease [94].

• The clinical reports demonstrated that infliximab is effective with rapid onset of action that gives the improvement with in days in crohns disease but it is not effective in ulcerative colitis treatment [95,96].

The onset of action of therapy is most important in the treatment of IBD especially in the treatment of ulcerative colitis. Although novel biological agents, immune modulator and other novel agents are available, therapy is dominated by amino salicylates and corticosteroids. The importance of onset of action in IBD or UC therapy determines the efficacy of therapeutic agents and therapeutic stratergies.

4.3.7 Advanced drug delivery systems in IBD

• Targeting the drugs to the colon gaining the importance in treating GI disorders. The local disorder such as IBD, irriTable bowel syndrome (IBS), carcinoma can be successfully treated by colonic deliveries.

• The advanced drug delivery systems prepared for the treatment of IBD mainly based on pH, transit time and microflora activation.

• The coated systems with pH dependent polymers such as polymethacrylic acid derivatives (Eudrgits) are widely used for this purpose [97]. Polysachhrides such as Pectin, Chito-

san, amylose andGuar gum can be successfully explored as colon drug delivery systems. The polysaccharide systems are found to be more successful because of their practicality and the abundant microflora of the colon.

• By combining the knowledge of threshold pH of the polymer and their solubility in different pH environments, duly, the system has been designed to release the drugs at target site exclusively on the colon[98,99].

• According to the various studies worldwide revealed and suggestedfluctuation in the pH of the colon is dueto various reasons [100].

• Some reports suggested that change in the G.I profile may occur in patient with IBD, which should be considered in developing delayed release formulations [101].

• Apparently the colon has lower pH value (6.5) than the small intestine (pH 7.0-7.8). The behavior of various pH sensitive polymers coated marketed products (Pentasa®, Asacol®, Salofalk®) with human subjects indicated that there was a marked individual variationin urinary recoveryof these drugs [102].

Table 2: Various Marketed Products of Mesalamine

Drug	Marketed product	Polymers (or) technology used	Site of release
Mesalamine	Asacol	Eudrgit S coating dissolves at pH > 7	
	Mesren	Eudrgit S coating dissolves at pH > 7	Distal part of intestine and colon
	Salofalk	Eudrgit L coated Tabletdissolves at pH > 6	Middle and Distal part of intestine and colon
	Pentasa	Ethyl cellulose coated granules membrane controlled release	Stomach to colon
Budesonide	Entocort	Eudragit L 100-55 coated ethyl cellulose granules dissolves at pH>5.5	Proximal intestine and colon
Sulfasalazine	Salazopyrine	Azo bond cleaved by colonic bacteria	Colon
Olsalazine	Pipentum	Two Mesalamine azo bond cleaved by colonic bacteria	Colon
Balsalazide	Colazide	Mesalamine with inert azo bond carries cleaved by colonic bacteria	colon

• Whenobservedin patients after administration of pentasa®, Tablets there was an individual variation in urinary recovery [103].

• Mesalamine Tablets manufactured by different companies have a different release profile when tested in various pH media.

• Research reports haveclearly stated that the pH sensitive polymer-based colonic deliveries may not release the drug in the colon as expected.

• There are possibilities of the drug being released in advance, prior toentry of the terminal part of the G.I tract and or poor cumulative percentage of drug release occuring due to variation in the colonic pH during IBD [104] Various studies proved that there was a fall in colonic pH in the IBD diseased colon [105].

5. Conclusion

IBD therapy is challenging to treat but the recent development in the drug delivery approaches demonstrating the promising results. The discussed drug delivery systems are reported by several researchers with encourage outcome in clinical and pre-clinical studies. Some drug delivery techniques are already in the market and offers the alternate ways to physicians to treat IBD. Nanomedicine or nanoparticulate drug delivery-based systems are emerging as an advanced approach to treat IBD in future. The nanoparticulate drug delivery systems may contribute more successrate in IBD therapy than the existed system in future due to their unique mechanism in drug delivery and reduced side effects. Therapy and drug delivery systems alone can't cure any disease without the support of life style changes and diet. Diet and life style changes has to be considered seriously before the occurrence of any disease rather than late.

6. References

1. Loyd V Allen, Nicholas G Popovich, Howard C Ansel, Ansel's Pharmaceutical Dosage forms and Drug delivery system, 8th edition, Lippincott Williams and Wilkins, 2010, 263-272.

2. Mithal BM, A Text book of pharmaceutical formulations, 6th edition, Vallabh prakashan, 2005, 147-347.

3. Abdul W Basit, Advances in Colonic Drug Delivery, Drugs, 65 (14), 2005, 1991-2007

4. Chourasia M K, JainSK, Pharmaceutical Approaches to Colon Targeted Drug Delivery System. Pharm Pharmaceutics Sci, 6(1), 2003, 33-66

5. ViolaAndresenand Michael Camilleri. Irritable Bowel Syndrome, Recent and Novel Therapeutic Approaches, Drugs, 66 (8), 2006, 1073-1088.

6. ALF Lamprecht, Nathalie Ubrich, Hiromitsu Yamamoto, Ulrich Schafer, Hirofumi Takeuchi, Philippe Maincent, Yoshiaki Kawashima, and claus, Michael lehr, Biodegradable nanoparticles for Targeted drug delivery in Treatment of Inflammatory Bowel Disease. The journal of pharmacology and experimental therapeutics, 299(2), 2001, 775-781.

7. Hiroshi Nakase, Kazuichi Okazaki, Yasuhiko Tabata, SuguruUose, Masaya Ohana, Kazushige Uchida, Toshiki Nishi, Andra's Debreceni, Toshiyuki Itoh,Chiharu Kawanami,MasaoIwano, Yoshito Ikada and Tsutomu Chiba. An Oral Drug Delivery System Targeting Immune-Regulating Ameliorates Mucosal Injury in Trinitrobenzene Sulfonic Acid-Induced Colitis. The journal of pharmacology and experimental therapeutics, 297 (3), 2001, 1122-1128.

8. Ishibashi, T., Hatano, H., Kobayashi, M., Mizobe, M. and Yoshino, H., Design and Evaluation of a new Capsule-Type Dosage Form for Colon-Targeted Delivery of Drugs, Int J Pharm, 168, 1998, 31-40.

9. Gupta, V.K., Beckett, T.E. and Price, J.C., A Novel pH- and Time-BasedMulti-Unit Potential Colonic Drug Delivery System. I. Development. Int J Pharm., 213 (1-2) 2001, 83-91.

10. Muraoka, M., Kimura, G, Zhaopeng, H. and Takada, K.., Ulcerative Colitis-Colon Delivery of 5-Aminosalicylic Acid. Nippon Rinsho., 56, 1998, 788-794.

11. Takaya T, Niwa, K., Muraoka M., Ogita I., Nagai N., Yano, R, Kimura G, Yoshikawa, Y, Yoshikawa, H. and Takada, K, Importance of Dissolution Process on Systemic Availability of Drugs Delivered by Colon Delivery System. J Control Rel., 50, 1998, 111-122.

12. Wirtz S., Galle P.Rl, Neurath M.F., Efficient Gene Delivery to the Inflamed Colonby Local Administration of Recombinant Adenoviruses with Normal or Modified Fibre Structure. Gut. 44 (6), 1999, 800-807.

13. Xing Mei Duan , Pan Wang , Ke Men , Xiang Gao , MeiJuan Huang MaLing Gou, LiJuan Chen , ZhiYong Qian and YuQuan Wei. Treating colon cancer with a suicide gene delivered by self-assembled cationic MPEG–PCL micelles. Nanoscale, 4, 2012, 2400-2407 .

14. Kopecek J, The Potential of Water -Soluble Polymeric Carriersin Targeted and Site-Specific Drug Delivery J Control Rel,11, 1990, 279-290.

15. Brondsted, H., Kopeckova, P., Hydrogels for Site-Specific Oral Drug Delivery: Synthesis and Characterization. Biomaterials. 12, 1991, 584-592.

16. Brondsted, H. and Kopecek, J., Hydrogels for Site Specific Drug Delivery to Colon: *Invitro* and Invivo Degradation, Pharm Res., 9, 1992, 1540-1545.

17. Gliko-Kabir, I., Yagen, B., Penhasi, A. and Rubinstein, A., Phosphated Crosslinkeid Guar gum for Colon-Specific DrugDelivery.I. Preparation and Physicochemical Characterization. J Control Rel., 63, 2000, 121-127.

18. Shanta K L., Ravichandran, P. and Rao K P., Azo Polymeric Hydrogels for Colon Targeted Drug Delivery. Biomaterials.,16, 1995, 1313-1318.

19. Vandelli M A, Leo E, Form F and Bernatei M T, *In vitro* Evaluation of Potential Colonic Drug Delivery System that Releases Drug after a Controllable Lag-Time. Eur J Pharm Biopharm., 43, 1996, 148-151.

20. Hovgaard L, Brondsted H, Dextran Hydrogels for Colon-Specific Drug Delivery. J Control Rel, 36, 1995, 159-166.

21. Ghandehari, H., Kopeeekova, P. and Kopccek, J., *In vitro* Degradation of pH-Sensitive Hydrogels Containing Aromatic Azo Bonds. Biomaterials., 18, 1997, 861-72.

22. Ulbrich, K., Strohalm, J. and Kopecek, J., Polymers Containing Enzymatically Degradable Bonds. VI. Hydrophilic Gels Cleavableby Chymotrypsin. Biomaterials, 3, 1982, 150-154.

23. Krogars K, Heinamaki, J, Vesalahti J. Marvola M., Antikainen O, Yliruusi J, Marvola M, Yliruusi J, Extrusion-Spheronization of pH-Sensitive Polymeric Matrix Pellets for Possible Colonic Drug Delivery. Int J Pharm, 199, 2000, 187-94.

24. Pomerantsev A L, Rodionova O Y, Melichar M, Wigmore A J, Bogomolov A. In-line prediction of drug release profiles for pH-sensitive coated pellets.Analyst. 136(22), 2011, 4830-8

25. Milojevic S, Newton J M, Cummings J H, Gibson G R, Botham R L, Ring, S C, Stockham M, Allwood M C, Amylose as a Coating for Drug Delivery the Colon: Preparation and *In vitro* Evaluation Using Glucose Pellets. J Control Rel., 38, 1996, 85-94.

26. Hovgaard L, and Brondsted H, Dextran Hydrogels for Colon-Specific Drug Delivery. J Control Rel., 36, 1995, 159-

166.

27. Wakerly Z, Fell J T, Attwood D, Parkins D, Studies on Drug Release from Pectin / Ethyl Cellulose Film-Coated tablets: A Potential Colonic Delivery System. Int J Pharm., 153, 1997, 219-224.

28. VyasS P and KharR K,Targeted and controlleddrugdelivery, 1stedition., CBS publishers and distributors, New Delhi, 2004, 417-425.

29. Willoughby C P, Aronson J K, Agback H, Bodin N O, Anderson E Truelove S C, Disposition in Normal Volunteers of Sodium Azodisalicylate, A Potential Therapeutic Agent in Inflammatory Bowel Disease. Gut. 22, 1981, A431.

30. Lauristein K, Hansen J, Ryde H M. Rask-Madsen J, Colonic Azodisalicylate Metabolism Determined by in vivo Dialysis in Healthy Volunteers and Patients with Ulcerative Colitis. Gastro¬enterology. 86, 1984, 1496-1500.

31. Azad Khan A K, Truelove S C, Aronseq J K, The Disposition and Metabolism of Sulphasalazine (Salicylazo-sulphapyridine) in Man. Br J Clin Pharmacol., 13, 1982, 523-528.

32. Jinhe Li, Libo Yang, Sheila M. FergusonTom J. HudsonShunsuke W, Masataka K, Joseph A. Fix. *In vitro* evaluation of dissolution behavior for a colon -specific drug delivery system (CODES™) in multi-pH media using United States Pharmacopeia apparatus II and III. AAPS PharmSciTech, 3 (4), 2002, 59–67.

33. Goh J, O'Morain CA. Review article: nutrition and adult inflammatory bowel disease. Aliment Pharmacol Ther., 17(3), 2003, 307-20.

34. Richard S. Blumberg, Warren Strober, Prospects for Research in Inflammatory Bowel Disease. JAMA., 285(5), 2001; 643-647.

35. Daniel K Podolsky, Inflammatory Bowel Disease, N Engl J Med, 347 (6), 2002, 417-429.

36. http://www.bcm.edu/medicine/ibd/infodiagnostic, 25/10/2012.

37.William A Rowe, Julian Katz, Inflammatory bowel disease medication, http://emedicine. medscape.com/article/179037- medication, last updated 13/09/2012.

38. Fallingborg J. Intraluminal pH of the human gastrointestinal tract. Dan Med Bull., 46, 1999; 183–96.

39. Fallingborg J, Pedersen P, Jacobsen BA. Small intestinal transit time and intraluminal pH in ileocecal resected patients with Crohn's disease. Dig Dis Sci.,43, 1998; 702–5

40. Raimundo A H, Evans D F, Rogers J, et al. Gastrointestinal pH profiles in ulcerative colitis. Gastroenterology,102, 1992; A681.

41. Watson B W, Meldrum SJ , Riddle H C. pH profile of gut as measured by radiotelemetry capsule. BMJ., 2, 1972; 104–6.

42. Evans D F, Pye R, Bramely, A G, Clark T J, Dyson J D. Measurement of gastrointestinal pH profiles in normal ambulant human subjects, Gut. 29, 1988, 1035-1041.

43. Raimundo A H , Patil D H, Frost P G, Silk D B A, Effects of olsalazine and sulphasalazine on jejuna and ileal water and electrolyte absorption in normal human subjects. Gut, 32, 1991, 270-274.

44. Fallingborg J, Christensen L A, Jacobsen B A, Ramusen S N. Very low intraluminal colonic pH in patients with active ulcerative colitis. Dig. Dis. Sci., 38: 1993: 1989- 1993

45. Nugent S G, Rampton D S, Kumar D, et al. Gut pH and transit time in ulcerative colitis appear sufficient for complete dissolution of pH dependent 5-ASA containing capsules. Gut.,47: 2000, (abstract)

46. Press A G, Hauptmann I A, Hauptmann L, et al. Gastrointestinal pH profilesin patients with inflammatory bowel

disease. Aliment Pharmacol Ther., 12, 1998; 673–8.

47. Ewe K, Schwartz S, Petersen S, et al. Inflammation does not decrease intraluminal pH in chronic inflammatory bowel disease. Dig Dis Sci., 44, 1999, 1434–9.

48. Sasaki Y, Hada R, Nakajima H, et al. Improved localizing method of radiopill in measurement of entire gastrointestinal pH profiles: colonic luminal pH in normal subjects and patients with Crohn's disease. Am J Gastroenterology., 92, 1997, 114–18.

49. Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. J Allergy Clin Immunol., 86, 1990; 202-10.

50. Rigaud D, Cosnes J, Le Quintrec Y, Rene E, Gendre JP, Mignon M.Controlled trial comparing two types of enteral nutrition in treatment of active Crohn's disease: elemental versus polymeric diet. Gut., 32, 1991, 1492-7.

51. Wallace JL, Nitric oxide-releasing Mesalamine: potential utility for treatment of inflammatory bowel disease Digestive and Liver Disease, 35 (Suppl. 2), 2003, S35–S40.

52. Green J R, Mansfield J C, Cann P A, A double blind comparison of balsalazide ,6.75gdaily and sulfasalazine 3g daily in patient with newly diagonised or relapsed active ulcerative colitis.Aliment Pharmacol Ther, 16(1), 2002, 61-68.

53. Mansfield J C, Giaffer M H, Cann P A, et al, A double blind comparison of balsalazide,6.75g, and sulfasalazin 3g, as sole therapy in the management of ulcerativecolitis. Aliment Pharmacol Ther, 16 (1), 2002, 69-77.

54. Green J R, Lobo A J, Holdsworth C D, et al. balsalazide is more effective and better tolerated than Mesalamine in the treatment of acute ulcerative colitis: the abacus investigator group. Gastroenterology, 114 (1), 1998, 15-22.

55. Abdul W. Basit - Advances in Colonic Drug Delivery, Drugs, 65(14), 2005, 1991-2007 .

56. Newton A M J, Prabakaran L, Jayaveera KN, Effect of luminal pH changes on Guar gum-HPMC E15 LV mixed matrix tablets for Mesalamine drug delivery to colon and study on *in vitro* charecteristics in two different dissolution models Vs marketed formulations, Int J Pharm Sci res, 3(7), 2012, 2337-2347.

57. Franchimont D, Kino T, Galon J, et al. Glucocorticoids and inflammation revisited: the state of art. NIH Clinical staff conference. Neuroimmuno modulation 10 (5), 2003, 247-60.

58. Faubion W A Jr, Loftus E V Jr, Harmsen W S, Zinsmeister A R, Sandborn W J. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. Gastroenterology , 121, 2001, 255-60.

59. Thomsen O, Cortot A, Jewell D, et al. A comparison of budesonide and Mesalamine for active Crohn's disease. N Engl J Med 1998 339:370-4. Erratum, N Engl J Med, 345: 2001; 1652.

60. Angus P, Snook J A, Reid M, etal. Oral fluticasone propionate in active distal ulcerative colitis. Gut, 33 (5), 1992, 711-4.

61. Hawthorne A B, Record C O, Holdsworth C D, et al, Double- blind trial of oral fluticasone propionate V prednisolone in the treatment of active ulcerative colitis, Gut, 34 (1), 1993, 125-8.

62. Campieri M, Adamo S, Valpiani D, et al . Oral becometasone dipropionate in the treatment of extensive and left sided active ulcerative colitis : a multicentre randomized study. Aliment Pharmacol Ther, 17 (12), 2003, 1471-80.

63. Friend D R, Issues in oral administration of locally acting glucocorticosteroids in the treatment of inflammatory bowel disease.aliment pharmacol Ther, 12 (7), 1998, 591-603.

64. Lewis J D, Bilker W B, Brensinger C, Deren J J, Vaughn D J, Strom B L. Inflammatory bowel disease is not associated with an increased risk of lymphoma.Gastroenterology ,121, 2001, 1080-7.

65. Farrell R J, Ang Y, Kileen P, et al. Increased incidence of non-Hodgkin's lymphoma in inflammatory bowel disease

patients on immunosuppressive therapy but overall risk is low. Gut, 47, 2000, 514-519.

66. Rosenberg J L, Wall A J, Levin B, et al. A controlled trials of azathioprine in the management of chronic ulcerative colitis. Gasteroenterology, 69 (1), 1975, 96-99.

67. Hawthrone AB, Logan RF, Hawkey CJ. et al. Randomized controlled trial of azathioprine withdrawal in ulcerative colitis. BMJ, 305 (6844), 1992, 20-22.

68. Nielson O H, Vainer B, Rask- madsen J, Review article : The treatment of inflammatory bowel diseasewith 6-mercaptopurine or azathioprine.aliment Pharmacol Ther, 15(11), 2001, 1699-708.

69. Corominas H, Domenech M, Gonzalez D, et al. Allelic variants of the thiopurine S-methyltransferase deficiency in patients with ulcerative colitis and in healthy controls. Am J Gastroenterol, 95, 2000; 2313-7.

70. Colombel J F, Ferrari N, Debuysere H, et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. Gastroenterology, 118, 2000; 1025-30.

71. Lichtiger S, Present D H, Kornbluth A, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med 330, 1994, 1841-5.

72. Mastuda S, Present D H, Kornbluth A, etal. Mechanism of action of cyclosporine. Immunopharmacology, 47(2-3), 2000, 119-125.

73. D'Haens G, Lemmens L, Geboes K, et al. Intravenous cyclosporine versus intravenous corticosteroids as single therapy for severe attacks of ulcerative colitis. Gastroenterology, 120, 2001, 1323-9.

74. Stange E F, Modigliani R, Pena A S, Wood A J, Feutren G, Smith P R.

European trial of cyclosporine in chronic active Crohn's disease: a 12-month study. Gastroenterology, 1995, 109, 774-82.

75. Gummert JF, Ikonen T, Morris RE. Newer immunosuppressive drugs: A Review. J Am Soc nephrol, 10 (6), 1999, 1366-1380..

76. Fellermann K, Tanko Z, Herrlinger K R, et al, Response of refractory colitis to intravenous or oral tacrolimus (FK506). Inflamm bowel Dis., 8 (5), 2002, 317-324.

77. Baumgart D C, Widenmann B, Dignass A U, Rescue therapy with tacrlimus is effective in patient with sever and refractory inflammatory bowel disease. Aliment Pharmacol Ther, 17 (10), 2003, 1273-1281.

78. Feagan B G, Fedorak R N, Irvine E J, et al. A comparison of methotrexate with placebo for the maintenance of remission in Crohn's disease. N Engl J Med, 342, 2000, 1627-32.

79. Feagan B G, Rochon J, Fedorak R N, et al. Methotrexate for the treatment of Crohn's disease. N Engl J Med, 332, 1995; 292-7.

80. Oren R, Arber N, Odes S, et al. Methotrexate in chronic active ulcerative colitis: a double-blind, randomized, Israeli multicenter trial. Gastroenterology, 110, 1996, 1416-21.

81. Alfadhli A A, McDonald JW , Fegan B G. Metrotrexate for induction of remission in refractory crohn's. Cochrane Database Syst Rev, (1), 2003, CD003459.

82. Ten Hove T, Van Montfrans C, Peppelenbosch M P, Van Deventer S J. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. Gut, 50, 2002, 206-11.

83. Lugering A, Schmidt M, Lugering N, Pauels H G, Domschke W, Kucharzik T. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. Gastroenterology, 121, 2001, 1145-57.

84. Creed T J, Norman MR, Probert CS, etal, Basiliximab (anti CD25) in combination with steroid may bean effective new treatment for steroid resistant ulcerative colitis . Aliment Pharmacol Ther, 18 (1), 2003, 65-75.

85. Van Assche G, Dalle I, Noman M, etal. A pilot study on the use of humanized anti- interleukin-2 recceptor antibody daclizumab in active ulcerative colitis . Am J Gastroenterol, 98 (2), 2003, 69-76.

86. Sutherland L, Singleton J, Sessions J, et al. Double blind, placebo controlled trial of metronidazole in Crohn's disease. Gut, 32, 1991, 1071-5.

87. Rembacken B J, Snelling A M, Hawkey P M, Chalmers D M, Axon A T. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet, 354, l999, 635-9.

88. Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology, 119, 2000, 305-9.

89. Hartmann G, Bidlingmaier C, Siegmund B, Albrich S, Schulze J, Tschoep K, Eigler A, Lehr H A, Endres S., Specific type IV phopsphodiesterase inhibitor rolipram mitigates experimental colitis in mice. J Pharmacol Exp Ther, 292: 2000, 22–30.

90. Yue G, Sun F F, Dunn C, Yin K, Wong P Y, The 21-aminosteroid tirilazad mesylate can ameliorate inflammatory bowel disease in rats. J Pharmacol Exp Ther, 276, 1996, 265–270.

91. Krawiscz, J E, Sharon P, Stenson W F, Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 87, 1984, 1344–1350.

92. Jagtap A G, Shirke S S, Phadke A S, Effect of polyherbal formulation on experimental models of inflammatory bowel disease. J Ethnopharmacol, 90, 2004, 195–204.

93. Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subsetof antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. J Allergy Clin Immunol, 86, 1990, 202-10.

94. Slonim A E, Bulone L, Damore M B, Goldberg T, Wingertzahn M A, McKinley M J. A preliminary study of growth hormone therapy for Crohn's disease. N Engl J Med, 342, 2000, 1633-7

95. Present D H, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patient with Crohn's disease. N Eng J Med., 340 (18), 1999, 1398-405.

96. Nikolaus S, Raedler A, Kuhbacker T, et al. mechanism in failure of infliximab for Crohn's disease : the edinburg experience. Lancet, 356 (9240), 2000, 1475-1479.

97. Zahirul M, Khan I, Prebeg Z, Kurjakvic N. A pH- dependent colon targeted oral drug delivery system using meth acrylic acid copolymers. J. Controlled Release., 58, 1999, 215-222

98. Follonier N, Cole E T, Evolution of hot melt extrusion as a new technique for the production of polymer based pellets for sustained release capsule containing high loadings freely soluble drugs. Drug Dev IndPharm., 20, 1994, 1323-1339.

99. Ashford M, Fell J T. Attwood D, Sharma H, Woodhead P. An *in vitro* investigation into the suitability of pH- dependent polymers for colon targeting, Int J Pharm., 91, 1993, 241-245.

100. Evans D F, Pye R, Bramely A G, Clark T J, Dyson J D, Measurement of gastro intestinal pH profiles in normal ambulant human subjects, Gut., 29, 1988, 1035-1041.

101. Adkin D A, Kenyon C J, Lerner E I, et al. The use of scintigraphy to provide "proof of concept" for novel polysaccharide preparations designed for colonic drug delivery, Pharm Res., 14 (1), 1997, 103-7.

102.Rijk M C M, Van Schaik, J H M, Van T. Disposition of 5-amino salicylic acid delivering compounds. J. Gastroen-

terology., 23, 1988, 107-112.

103. Stolk, L M L, Rietbroek R, Wiltink E H, Tukker J J. Dissolution profile of mesalazineformuations *in vitro*. Pharm. WeekblSci, 12(5), 1990, 200-204.

104. Jose S, Dhanya K, Cinu TA, Litty J, Chacko AJ, Colon targeted drug delivery: different approaches. J Young pharmacist., 1, 2009, 13-19.

105. Nugent S G, Kumar D, Rampton D S, Evans D F. Amino salicylates and other drugs possible determinants and implications for therapy Intestinal luminal pH in inflammatory bowel disease, Gut, 48, 2001, 571-577.