Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Colon Drug Delivery Systems for the Treatment of Inflammatory Bowel Disease

Milad Reda Qelliny[*], **Omar Helmy Elgarhy, Khaled Aly khaled, Usama Farghly Aly**

Department of Pharmaceutics, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

Abstract

Inflammatory bowel disease (IBD) is one of the most common chronic diseases that affect the entire gastrointestinal tract (GIT) especially the colon. Its symptoms extend from mild diarrhea, abdominal pain, and bloody diarrhea to severe conditions which affect the quality of life. Many treatments have been developed to treat and cure IBD and to improve patient's quality of life. The big challenge faces the newly developed treatments is the site of action as the colon presents at the distal end of the GIT and have a complex biological environment. Many technologies have been investigated to target the colon, load higher amounts of active ingredients, and decrease unwanted side effects resulted from upper GIT absorption. This review briefly discusses the IBD, treatment lines, physiological considerations, and all methods of colon targeting technologies starting from the traditional methods which based on pH, time, and microbial content of the colon. Also, we discussed in detail all new techniques based on Micro and Nanotechnology which improve the effectiveness of used therapeutics.

1. Introduction

Newly designed pharmaceutical drug delivery systems focus on delivering existing drugs with improved safety and efficacy together with lower dose frequency [1]. Also, the choice of the most appropriate administration route is very important in order to achieve the required therapeutic response. [2]

In comparison with the alternative routes of drug delivery, oral route and oral delivery systems are considered to be most suitable and best to administer drugs. Oral route has many advantages above other routes such as easiness in administration, low cost, and patient noncompliance. [3] The main drawbacks and the most serious problem in the oral route and using conventional drug delivery systems are allowing the amount of active drug level in plasma devoid of any control over the delivery of active substance [4]. In addition, drug absorption from gastrointestinal tract (GIT) regions depends mainly on physicochemical properties of the active ingredient. [5]

Modified-release systems showed a controlled manner of the required plasma levels and steady-state concentration for a long period [4]. The advanced drug delivery systems planned to control drug release in the oral route also, planned to control the release of poorly water-soluble drugs and to target specific GIT sites [6].

Pharmaceutical researchers extensively studied and developed in the area of drug targeting and/or site-specific drug delivery. Delivery of drugs to specific sites or to treat specific diseases is very important and essential to improving therapeutic efficiency by increasing the dose of the desired drug at the site of action. Also, to reduce undesirable side effects and cost [7].

Colon drug delivery systems (CDDS) are an example of drug targeting which has promising developments in the area of local and systemic treatment. At the same time, CDDS have various challenges as reaching the distal part of GIT presents significant physiological difficulties and environmental barriers [8, 9]. Targeting drug to the colon is highly valuable for local treatment of numerous diseases such as ulcerative colitis, Crohn's disease and colonic cancer [10]. Also, for the systemic delivery of drugs such as proteins and peptides which may be unstable in the stomach and small intestine due to many problems like hydrolysis and lower absorption from the lumen of upper GIT due to their relatively large molecular weight [11].

2. Anatomical and physiological considerations related to the colon

The colon is the terminal part of the GIT. It is a part of the large intestine and has the following anatomical features:-

1. The length of the colon is about 1.5 – 1.66 m (5 ft).
2. Having an internal diameter of 2.5 cm and a surface area 3 m^2.
3. Starts from the ileum by a small junction called ileocecal sphincter and ends with the anus.
4. According to the anatomical structure, the large intestine is divided into four anatomical positions are cecum, colon, rectum, and anal canal.
5. The colon is divided into four regions are ascending colon, transverse colon, descending colon, and sigmoid colon [8, 12, 13].

* *Correspondence: Milad Reda Qelliny*
Tel.: +2 01228281799; Fax: +20 862369075
Email Address: mila_reda@mu.edu.eg

As the colon is the distal part of the digestive system, so having some particular physiological features rather than the upper GI tract as (**Figure 1**):-

1. Colon fluids:- colon have a 178 ml of total fluids and about 13 ml of free fluids, which are a very little amount of water to solubilize drugs [8] and considered a big challenge to drug absorption.
2. Microbial flora:- a large number of microorganisms occupy the colon, which approximately accounts and more than 3000 different species [14]. These microorganisms are able to digest many contents of the colon as polysaccharides, proteins, peptides, and drugs. Over than 30 drugs have been identified to be subjected to microbial digestion in the colon [15].
3. Transit time:- the colon shows a big variation in residence time; the residence time in the colon can be from around 1 hr up to several days [16] and this could affect drug absorption and subsequently affects drug bioavailability [17].
4. Digestion in the colon consists of two main mechanisms, the first one is mechanical digestion in which digestion starts when the chyme passes through the ileocecal sphincter; the characteristic movement of the colon is haustral churning in which colon walls contract and squeeze contents into the second haustrum. The final digestion mechanism is chemical digestion in which microorganisms that inhabit the lumen of the large intestine digest and ferment colon contents and release gases as carbon dioxide, two mechanisms are challenging drug absorption from the colon [12].

3. Inflammatory bowel disease (IBD)

Inflammatory bowel disease (IBD) is a relapsing and chronic inflammatory disease of bowel mucosa [18], more susceptible to the colon [19]. IBD is a chronic, progressive, disabling disease [20], characterized by the unknown origin and both long-term and short-term inflammation [21]. IBD is a term used to describe both ulcerative colitis (UC) and Crohn's disease (CD) [22-24]. Both diseases are thought to be a result of dysregulated mucosal response in the bowel function [25]. Both UC and CD are usually extending over many years and sometimes impossible to differentiate between them [23]. Whatever both are characterized by similar symptoms, for example, severe diarrhea, bodyweight loss, bloody stool and abdominal pain [23]. Pathological lesions and the position of the inflammation can distinguish between UC and CD to some extent [26]. In the case of UC, inflammation mainly affects the innermost mucosa and not involve the deeper tissues like serosa and muscularis. The lesion mainly is confined to the colon and rectum. But in case of CD, inflammation is transmural, affects the entire wall of the intestine, and deeper to the serosal layer. The lesion occurs over the length of the large intestine and small intestine, sometimes even reach to the mouth [27, 28]. IBD characterized by alternative cycles of remission and relapse [22, 29]. Although IBD has been extensively studied for many years, its pathogenesis remains idiopathic and unknown [30].

The pathogenesis for IBD (**Figure 2**) is to some level can be explained and understood, IBD is believed to occur due to dysregulation of the immune response to commensal microbiota in genetically susceptible individuals [26, 31]. Much clinical evidence consider dysbiosis of the intestinal microbiome with developing of UC and CD [22, 32]. IBD has both genetic and environmental risk factors [23]. Genetic related factors include i.e, the mutation of NOD 2 encoding genes and HLA*103 which associated with severe UC. Approximately 15 % of the patients with IBD have a first degree- relative to the disease, but the inheritance pattern of the disease is not clear [33, 34]. Many new studies discuss NOD 2 genes mutations which considered as the main driver of early onset of CD [35]. On the other hand, many environmental factors related to IBD and not clearly understood such as occupation, breastfeeding, oral contraceptives, stress, smoking, microbes, drugs, and diets [36-39].

Pathophysiology of IBD involves multiple complex pathways in the deregulation of the inflammatory cascade, which include increased intestinal permeability and lower intestinal barrier resistance of inflamed cells [40]. Mechanisms include bacteria taken up by specialized M-cells and enter the lamina propria through ulcerated mucosa [23], T-cell mediated disruption of tight junctions proteins [41, 42], increased levels of cytokines and interleukin 12 (IL-12) [43], resistance of activated T-cells to normal apoptosis, and finally, high response of T-cells to interferon γ (INF-γ) release [44].

4. Drug molecules for IBD treatment

Pharmacological treatment of ulcerative colitis and Crohn's disease is very difficult and depends mainly on the location and activity of the disease. The main goal of treatment is to prolong remission cycles and decrease relapsing cycles. IBD treatment is long-life treatment [45]. Wide range of medicinal agents used for the treatment of IBD as 5-aminosalicylates, glucocorticoids, antibiotics, thiopurines, methotrexate, and biological treatment as TNF-α antibodies [23].

5-aminosalicylates are the first line of the treatment for patients having mild to moderate UC and have a big role in induction and maintenance of remission periods at doses of 3000-4500 mg per day for sulfasalazine [26].

Aminosalicylates group include sulfasalazine [46], mesalazine [47], olsalazine [48, 49], and balsalazide [50, 51]. The action of aminosalicylates depends on the modulation of cytokines released from bowel mucosa [23]. Also, by decreasing the nuclear localization of nuclear factor-kappaB (NF-kB) through peroxisome proliferator-activated receptor gamma (PPAR-γ) mediation [24]. Aminosalicylates are the most common treatment of ulcerative colitis [24, 46], and have no proven role in the treatment of Crohn's disease [23]. Sulfasalazine having more side effects due to sulfapyridine-related intolerance in some patients [52], so the use of sulfasalazine is limited. Other agents as mesalazine, olsalazine, and balsalazide are more tolerated.
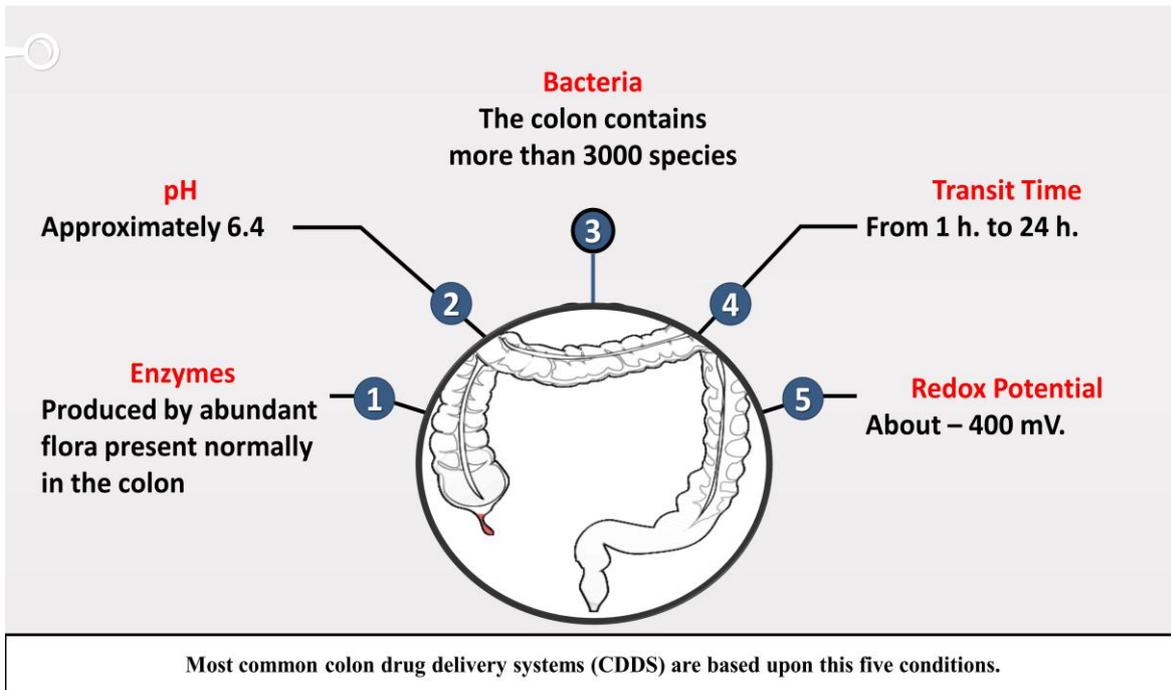
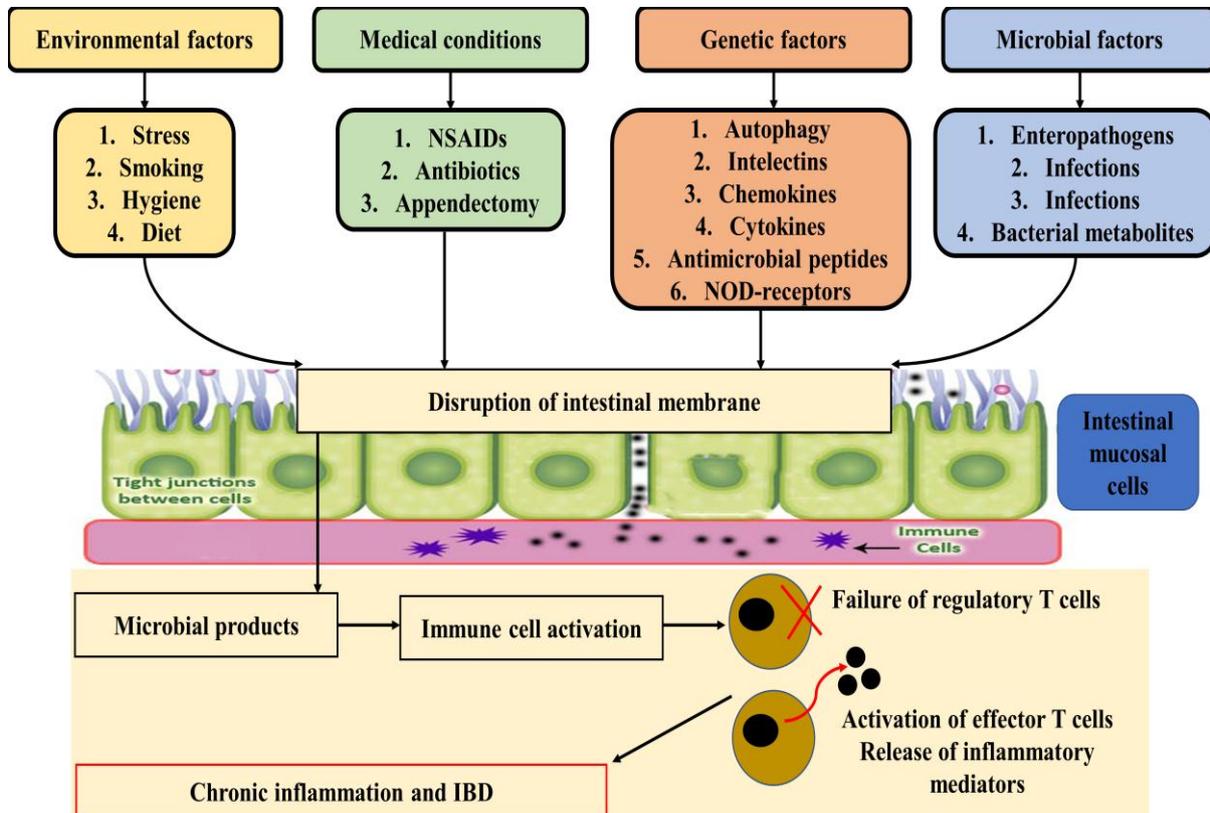**Figure 1:** The most important colon environment conditions.

Figure 2: Pathogenesis of inflammatory bowel disease.

Glucocorticoids are considered to be the first line for the treatment of active IBD based on clinical and experimental findings. About 54 % of patients with UC showed complete remission over one month of the treatment, 30 % showed partial remission and 16 % showed no remission. In CD patients, glucocorticoids treatment course for one month showed that 58 % of patients with complete remission, 26 % showed partial remission and 16 % of patients showed no response [53]. Glucocorticoids used in the treatment of inflammatory bowel disease include prednisolone [54, 55], hydrocortisone [23], and budesonide [23, 24, 56, 57]. Corticosteroids action depends mainly on the potent anti-inflammatory effect which characterizes this group. Generally, corticosteroids are effective in the treatment of moderate to severe ulcerative colitis and having no role in the maintenance treatment of both ulcerative colitis and Crohn's disease [24]. Administration of corticosteroids may be oral, intravenous or topical as an enema [23]. Budesonide is considered the potent corticosteroid, having fewer side effects [24] and used for the treatment of active disease especially ileitis, ileocolitis, and Crohn's disease.

In case of patients with severe IBD and do not respond to aminosalicylates or glucocorticoids treatment due to corticoid dependency and resistance [58] can be treated with immunosuppressive agents as methotrexate, cyclosporins, azathioprine [59] and mercaptopurine [60].

Targeting inflammatory cascade at the main point is considering a good feature in the treatment of IBD [61], targeting includes TNF-α which is a main inflammatory mediator and involved in many systemic and cutaneous inflammatory disorders [62]. In this case, antibodies used to neutralize TNF-α, the human chimeric monoclonal antibodies infliximab that binds to the soluble sub-unit and the membrane-bound precursor of TNF-α [63]. Other antibodies approved by the FDA and used for IBD treatment as adalimumab and certolizumab [24, 64].

5. Drug delivery strategies for IBD therapy

5.1. Colonic absorption

The colon has a different physiological environment and in the case of IBD, the colon environment becomes more complex due to the disease severity and location of the lesion in the distal part. Also, treatment becomes more difficult due to the previous conditions and due to colonic absorption, which hardly to be predicted as the small intestine. Irrespective of therapy required for local or systemic drug delivery, drug absorption from the colon mainly depends on three major factors include pH, transit time, and microbial flora of the colon [65]. The large intestine characterized by the small surface area 3 m2 [8]. The small surface area of the colon is overcompensated by the very long transit time (≤ 48 h.) [66] and the absence of digestive enzymes. Drugs which reached to the colon may be absorbed by two main mechanisms, the first one is the transcellular transport in which the drug passes through colonocytes, and the other is the paracellular transport in which drug passes through the junctions between adjacent colonocytes [67]. The paracellular pathway is highly difficult and more restricted in the colon due

to very small gaps between colonocytes-very tight junctions-only molecules of 60 molecular weight or lower can be absorbed paracellular. Absorption in the colon occurs by the second transcellular pathway (passive transcellular diffusion) in which lipophilic drugs pass through colonocytes but not similar to the small intestine as the colon having lower water volume and small surface area available for drug absorption [68].

Drug delivery strategies to the colon (**Figure 3**) include at the first rectal preparations like suppositories, enemas, and foams. Rectal preparations have been efficiently used for the treatment of lesions in the lower part of the colon, but not effective in some cases in which the inflammation was located in the upper part of the colon such as pancolitis [26]. The traditional oral route is considering an effective route for the treatment of IBD especially lesions that extend to the small intestine and ever to mouth. Oral route has many limitations as extensive first-pass metabolism, side effects due to drug absorption from upper GIT and only a small amount of the active drug reach to the inflamed areas of the colon.

5.2. Factors affecting drug absorption from colon

5.2.1. Drug related factors

Drug absorption from the colon differs from other sites of GIT as the colon is the distal part of the alimentary canal and having some different features. Also, drug properties affect drug absorption from the colon as [69, 70]:-

1. Drug solubility, drug log P, and permeability at the site of action [71].
2. Physicochemical properties of the drug as pKa and degree of ionization.
3. Drug degradation and stability in the colon [72].
4. The drug should be in solution before reaching the colon, where the water volume and fluids content is low [73].

5.2.2. Colon related factors

The colon environment has a big role in drug absorption by different and various factors which affect the absorption rate as [74]:-
1. Lumen pH level.
2. Transit time of the colon which has higher values and big variations.
3. Bacterial enzymes activity against drugs.
4. Mucous binding and selectivity to drugs.
5. Disease state of the colon.
6. Local physiological action of the drugs.

5.2.3. Formulation related factors

Colon targeted drug delivery systems should be formulated in a manner which produces the highest drug targeting and highest drug absorption from the colon. Many formulation factors can affect drug absorption as:-
1. Type of drug delivery system.

2. Polymer and excipients nature.

3. Drug delivery system release manner, which should be able to control release in the stomach, upper gastrointestinal tract, and able to release the drug in the colon [71] [75].

4. Particle size as microparticle or nanoparticle delivery systems.

5. Using of absorption enhancers.

6. Colon drug delivery systems (CDDS) should be able to delay drug release till reaching the colon, in which formulation may release the drug in burst manner or sustained-release [76].

7. Formulation factors, retention time, and retrograde spreading influence drug concentration reaches the colon [77].

5.3. Physiological consideration in colon drug delivery systems design

5.3.1. Transit time

A big variation in physiological state occurs in IBD patients and becomes dynamic, more inter-related, and difficult to examine correctly in isolation. Transit time across the gastrointestinal tract (Orocecal transit time, OCTT) has been shown to be delayed in both ulcerative colitis and Crohn's disease [22] [78]. Patients with ulcerative colitis have colonic transit time twice faster than normal persons due to high secretions and diarrhea, leading to challenges in targeting the colon using conventional formulations. OCTT in the normal and IBD patients shown in (**Table 1**). Using the delayed-release conventional formulations is not effective in colon targeting and showing bidistribution phase as higher drug concentration in the proximal colon and lower drug concentration in the distal colon [79].

5.3.2. Microbial contents

Normal flora occupies our gastrointestinal tract from mouth to the colon and plays a big role in GIT physiology as digestion of carbohydrates, proteins, and fatty acids. In normal conditions, the GIT hosts over 500 distinct species [22], and many studies estimating the number of species to 2000 [80]. Gastrointestinal microbiota is a complex system includes bacteria, yeasts, archei, and fungi [14]. The colon contains at least about 1011 - 1012 CFU of microorganisms and the most common types in the colon are *Bacteroids*, *Clostridium group IV, XIV*, *Bifidobacteria*, and *Enterobacteriace* [22, 80].

Ulcerative colitis and Crohn's disease occur in the colon and distal ileum, which having the highest concentration of microbiota. Both composition and function of intestinal microorganisms in UC, CD, and pouchitis are abnormal [80].

Dysbiosis is the imbalance of the normal microbial flora and considered as one of the common theories of IBD pathogenesis, in which occur an increase in the concentration of anaerobic bacteria, particularly gram-negative (G -Ve) bacteria as *Bacteroids*, and reduction in beneficial bacteria as *Bifidobacteria* [81]. Also, dysbiosis of commensal microbiota includes decreased the ratio of protective/ aggressive bacteria, decreased the microorganisms which produce short-chain fatty acids (SCFA), and increased the concentration of aggressive bacterial species as hydrogen sulfide reducing bacteria, *Bacteroids*, *Enterobacteriace*, and *Candida albicans* [80]. Normal microbial flora and dysbiosis are presented in (**Table 2**).

5.3.3. Colonic pH

Gastrointestinal pH changes along different regions of alimentary canal as shown in (**Table 3**). The highly acidic stomach secretions and contents rapidly changed to slightly acidic pH in the duodenum and then rose to basic pH at the terminal ileum [22, 82].

The colon pH in normal individuals changes from cecal pH of 6 to the rectum pH of 6,7 [71, 83]. The slightly acidic pH of the colon is due to the production of short-chain fatty acids (SCFA) by the abundant bacterial microbiota of the colon [84]. The gastrointestinal pH controlled by many factors as the food and fluid intake, microbial digestion and fermentation process, and gastrointestinal secretions [85]. During the active phase of inflammatory bowel disease occurs disruption in three main mechanisms which control luminal pH level, microbial fermentation and digestion process especially SCFA production in the colon, bile acid metabolism of fatty acids, and bicarbonate/carbonate secretions mechanism [82]. Disruption of these mechanisms leads to alterations in the colon pH from 6,8 to 5,5 in active UC, [71] and 5,3 in CD [22, 83]. Alterations in pH lead to a change in transit time and microbial flora contents, which significantly affects drug release from traditional formulations [86].

5.3.4. Intestinal membrane integrity

Normal intestinal barrier composed mainly of the following three layers:-

1. Thick mucus layer, which composed of two main layers, the outer mucus layer, and the inner mucus layer. Mucus produced by goblet cells consisting of a thick layer of about 150 μm and acts as a chemical barrier by protecting the intestinal epithelium by its viscosity. Mucus layer contains a high concentration of glycosylated mucins, and trefoil factors (TFFs), which acts as a defensive mechanism. Also, acting by entrapping bacteria [87].

2. A Monolayer of epithelial cells, which mainly composed of colonocytes, and goblet cells. The epithelial cells regulate the intestinal permeability between the cells by junctions, the most common types of colonocytes junctions are desmosomes, adherent junctions (AJs), and tight junctions (TJs) [87] [(88, 89].

3. The lower barrier, which composed mainly of a group of cells as macrophages, mesenchymal cells, dendritic cells, and lymphocytes. Thes layer acts mainly as a protective layer.

Chronic inflammation of intestinal membrane as in both UC and CD leads to destructive changes in the intestinal barrier as:-

1. Disruption of intestinal membrane characterized by mucosal surface changes and crypt distortion [22].
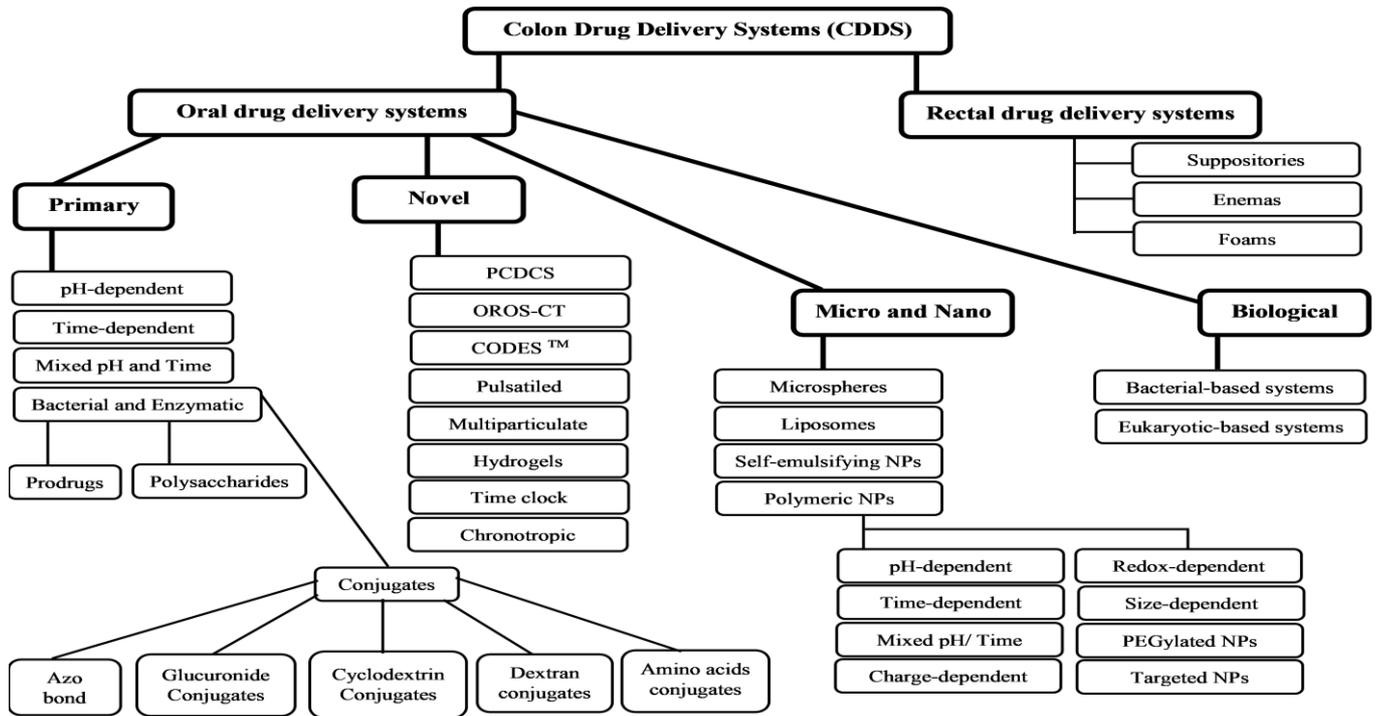
Figure 3: Colon-targeted drug delivery systems.

Table 1: OCCT in normal individuals and IBD patients.

Transit time (Hours)	Normal	IBD
1. Stomach.	1 – 2 hr.	Increased 30%
2. Small intestine.	3 – 4 hr.	Increased 30%
1. **Duodenum**	2 hr.	
2. **Jejunum**		
3. **Ileum**	1.5 hr.	
	1.5 hr.	
3. Large intestine.	6 – 70 hr.	24 hr.

Table 2: Commensal microbial content of gastrointestinal tract in healthy individuals and IBD.

GIT parts	Microorganism count	Species in healthy	Species in IBD
1. stomach	10^2	*Clostridiales*	Increased *E.coli.*
2. small intestine		*Streptococcus*	Decrease *Clostridium*
1. duodenum	10^4	*Bacteroids*	
2. jejunum	10^5	*Actinomycinae*	
3. ileum	10^7	*Lactobacillus*	
		Corynebacteria	
3. colon	10^{11}	*Firmicutis*	Increase *bacteroids*, *Eubacteria*,
		Bacteroids	*Peptostreptococcus*, and decrease
		Proteobacteria	*Bifidobacteria* and *E.coli.*
		Actinobacteria	

2. Reduction in a number of goblet cells, reduction in mucus production, reduced mucus layer thickness, and altered mucus composition [90].

3. Infiltration of inflammatory immune cells as lymphocytes, neutrophils, and macrophages.

4. Changes in mucosal physiology and metabolism, as membrane trying to repair and limit damage of cells, the compensation mechanism leads to activation of a number of protective pathways as the oxygen-sensing transcription factor, and hypoxia-inducible factor (HIF) mediates increased expressions of mucus components as mucins, and TFs, subsequently leading to mucus viscosity changes, which may affect permeability of lipophilic drugs [91].

5. Changes in mucosal membrane transport mechanisms as downregulation of TJ complex, which associated with loss of intestinal integrity [22], and increased paracellular absorption in patients with IBD [92]. TJ complex is considered as an attractive target for drug absorption [93]. Also, HIF transcriptionally regulates multi-drug resistance gene 1 (MDR 1), which stimulate both xenobiotic drug efflux pump, and P-glycoprotein (P-gp), which actively acting in the transportation of the drug back again to the lumen, and contributes to many drug resistance, For example, glucocorticoids [94, 95].

5.4. Primary approaches for colon drug delivery

Main strategies for the colon drug delivery systems include primary or traditional approaches such as tablets which mainly depends on three main mechanisms namely, enzymatic or microbial approach which mainly acts by the aid of colonic microbial enzymes, pH-dependent approach, and time-dependent approach.

In microbial or enzymatic approach, targeting depends mainly on drug activation by colonic microbial enzymes. The colon contains at least about 1011 - 1012 CFU of microorganisms and the most common types are *Bacteroids*, *Clostridium group IV, XIV*, *Bifidobacteria*, and *Enterobacteriace*. [22, 80] The main drawbacks of this system are its dependency on the enzymatic activity of colonic normal flora that may be totally disrupted in case of IBD. Dysbiosis, which is defined as the imbalance of the normal microbial flora and considered as one of the common theories of IBD pathogenesis, is not common in case of UC, but in CD many variations in microbial enzymes have been observed [96, 97]. The microbial-based approach includes using of prodrugs, the most common example is sulfasalazine and 5-ASA which cleaved microbially and activated to mesalazine and sulfonamide [98, 99]. Also, include the use of conjugates like azo-bond conjugates, glucuronide conjugates, cyclodextrin conjugates, dextran conjugates, and amino acids conjugates [69, 100-102]. Finally, this system is widely available using a variety of polysaccharides (**Table 4**).

In the pH-dependent approach, a widely used approach and depends mainly on the retardation of drug release at lower pH values. Therefore, drug release occurs only at pH of distal ileum (pH > 6). Patients with IBD showed lower colonic pH ranging from 5 to 7 and in some cases drops to 2.3 which cause incomplete drug release at the site of treatment [71, 82].

Time-dependent systems or time-controlled systems are usually known as delayed-release systems or sigmoidal-release systems [103]. The system is designed mainly to resist the acidic medium of the stomach, prevent drug release in the upper GIT, and unaffected by the intestinal bacteria or enzymes [70, 104, 105]. The main drawbacks of time-dependent approach may be concluded in the following: the gastric emptying time is variable, inconsistent between individuals and depends mainly on food intake, type of food, size, shape, the density of the dosage form, disease conditions, and gastric motility associated with the physiological condition of the patient [105-111]. The release of the drug from time-dependent systems occurs by different mechanisms such as swelling mechanism, osmosis mechanism or combination of both [104, 112]. Erodible polymers (**Table 5**) are most common used for time-controlled systems as a lag time can be built in it to allow drug release from the dosage form after this time, such as Eudragit RS 100, Eudragit RL 100, hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), and hydroxyethyl cellulose (HEC) [5, 9, 113].

5.5. Novel drug delivery systems

5.5.1. Pressure controlled drug delivery systems (PCDCS)

The large intestine has more peristaltic movements than the small intestine producing a higher-pressure property. Taking into consideration this point, Takaya *et al.* [114] developed a new technique depends on the pressure difference between the small intestine and the colon. The new drug delivery system is based mainly upon the using of ethyl cellulose which is a water-insoluble polymer. The system is composed mainly of a drug containing capsule covered with ethyl cellulose polymer. The drug release is controlled by the disintegration of the polymer due to the pressure inside the lumen of the colon. The main driving parameter controlling the drug release is the thickness of the capsule shell [10, 72, 108, 115, 116].

5.5.2. Osmotic controlled drug delivery systems (OROS-CT)

Generally, osmotic based drug delivery systems are very common drug carriers in the oral route. The system mainly designed upon the difference in the osmotic pressure generated between the system and the lumen of the colon. The colon has osmolarity of 81 mOsm/Kg, which is the main driving force affecting the drug release from the osmotic based systems. This system is designed to target and treat colon conditions like IBD or to attain drug release for many drugs that degraded in the small intestine. The OROS-CT may be composed of one unit or 5-6 push-pull units, encapsulated within a hard gelatin capsule. The main composition of osmotic based drug delivery carriers is the main unit which containing osmotic drug compartment and osmotic push compartment covered with a semipermeable membrane with a small orifice drilled through the drug

Table 3: Gastrointestinal luminal pH in Healthy individuals and IBD patients.

GIT parts	Normal pH	IBD pH
1. Stomach	1.5	
1. fed state	3 – 5	
2. fasted state	1.5 – 2.0	
2. Small intestine		
1. Duodenum	6	7.4
2. Jejunum	6.8 – 7	7
3. Ileum	7.4	7.4
3. Colon		
1. Ascending colon	6 – 8 (6.4)	2.3 – 6.5
2. Transverse colon	6 – 8 (6)	2.3 – 6.5
3. Descending and sigmoid	6.7	2.3 – 6.5

Table 4: Polysaccharides used for colon drug delivery.

No.	Polysaccharide	Properties	Bacteria species that degrade the polymer.
1	Amylose	Unbranched ingredient of starch	Bacteroids
2	Arabinogalactan	Natural pectin	Bifidobacterium
3	Chitosan	Deacetylated chitin	Bacteroids
4	Dextran	Plasma expanders	Bacteroids
5	Chondroitin sulfate	Mucopolysaccharide contains sulfate ester	Bacteroids
6	Cyclodextrin	Cyclic structure of 6,7, and 8 units	Bacteroids
7	Guar gum	Galactomannan, thickening agent	Bacteroids and Ruminococous
8	Pectin	Partial methyl ester, thickening agent	Bacteroids Bifidobacterium Eubacterium
9	Inulin	polysaccharide composed of a mixture of oligomers and polymers	Bifidobacterium
10	Xylan	Abundant hemicellulose	Bacteroids
11	Chitosan derivatives	Chitosan succinate and phthalate	Bacteroids
12	Locust bean gum	Mainly galactomannan units	Bacteroids

compartment. The entire unit is covered with an enteric impermeable membrane (**Figure 4**).

The mechanism of drug release from osmotic based systems could follow the following cascade; first, the gelatin capsule dissolves immediately after the system is swallowed. The entire system is covered with an impermeable membrane which resists drug release at the acidic pH of the stomach. Secondly, at the higher pH of the intestine (pH > 7) the semipermeable membrane starts to dissolve, and the water enters to the central unit causing the osmotic push compartment to swell and creates a flowable gel in the drug unit. Finally, the swelled osmotic push unit forces the drug gel out of the orifice, and the drug release occurs at a controlled manner and over a precise time [10, 72, 108, 115, 116].

5.5.3. A novel colon targeted system (CODES™)

A new technique was developed to overcome the drawbacks of the pH and time-dependent drug delivery systems. The CODES™ system (**Figure 5**) is mainly composed of a simple tablet core containing the active ingredient and coated with acid-soluble polymer and a degradable polysaccharide such as lactulose layer, then a new layer of the enteric polymer Eudragit L 100 or hydroxy methylcellulose (HPMC) polymeric coat is added and finally the tablet was coated with Eudragit E polymer. The enteric polymer protects the system inside the stomach and until the system delivered to the small intestine. At the higher pH of the small intestine, the enteric coat starts to dissolve with the presence of barrier layers such as HPMC or Eudragit L 100 to prevent the interaction between polymeric coats. At the colon, lactulose starts to dissolve by the aid of microflora producing a sufficient acid media capable for dissolving the acid layer surrounding the drug and affect the drug dissolution rate [10, 72, 108, 115, 116].

5.5.4. Pulsatile drug delivery system (PulsinCap®)

Simply, the system is mainly based on the time-dependent approach and the PulsinCap® is the most common one. The new technology composed of insoluble half capsule body filled with an active ingredient, the open end of the capsule sealed with a fixed amount of hydrogel plug, the plug coated with water-soluble cap, and finally, the whole capsule coated with an enteric polymer film (**Figure 6**). The capsule is resistant to various degradation processes in the stomach and the polymeric coat starts to dissolve at higher pH of the small intestine. The plug composed of semipermeable materials which permit water transfer to the drug compartment. The length of the fixed plug controls the rate of drug release from the system [10, 72, 108, 115, 116].

5.5.5. Multiparticulate drug delivery systems

A multiplicity of small discrete units such as pellets, granules, beads, microparticles, or nanoparticles filled into a sachet or compressed into a tablet matrix. In these dosage forms, the system able to escape from the upper gastrointestinal degradation due to their relatively small size. Lower and uniformity of the particle size ensure more uniform GIT dispersion and uniform drug release manner. The main advantage of this system is lower inter and intra-subject variability in gastrointestinal transit time as the smaller particle size is less dependent on the gastric emptying time [66, 116].

5.5.6. Hydrogels drug delivery systems

A network of materials capable of absorbing water but remaining insoluble and mainly formed by two mechanisms: covalent crosslinking of linear hydrophilic polymers and heterogeneous polymer mixtures. The most common hydrogels available for colon targeting purpose are mainly based on azo polymeric networks such as inulin, polyvinyl alcohol, guar gum, and dextran [72, 104, 107, 108, 116, 117].

5.5.7. Time clock-based drug delivery systems

The new technology designed to release the drug at the colon and after a specific time. The system composed mainly of solid dosage forms such as tablets or capsules and covered with a hydrophobic surfactant layer. Finally, an outer coat of water-soluble polymer is added to increase adhesion to the core. The outer coat disperses in the aqueous media of the GIT in a time proportional to the thickness of the coat. After total redispersion of the coat, the core is then available for redispersion and drug release starts. Many studies showed that the lag time is independent on the digestive enzymes, and the mechanical action of the stomach [72, 104, 107, 108, 116, 117].

5.5.8. Chronotropic drug delivery systems

An oral drug delivery system is used to target site-specific diseases such as IBD. Chronotropic systems (**Figure 7**) are mainly designed to achieve time-dependent drug release. In general, a drug-containing reservoir coated with a water-soluble polymer like HPMC, and the final coat is a gastroprotective polymeric film, which is responsible for the drug-resistant to the degradation in the stomach. The polymeric film starts to dissolve at higher alkaline pH of the small intestine, and the drug release lag time is dependent on the thickness of the water-soluble coat and the viscosity of the polymer used [72, 104, 107, 108, 116, 117].

### 5.5.9. Other novel drug delivery systems

A wide range of newly designed colon drug delivery systems have been evaluated in the last decade to enhance colon-specific drug targeting. For example, bioadhesive-based systems using various polymers such as polycarbophils, and polyurethanes, redox-based systems, COLAL® tableting technology, MMX® technology, and PHLORAL® technology (**Table 6**).

Table 5: Enteric polymers investigated for colon-based drug delivery systems.

| No.	Polymer	Properties	pH or time dissolution threshold
	A. pH-sensitive polymers:		
1	Eudragit L 30 D-55	30% aqueous dispersion	Above pH 5.5
2	Eudragit L 100-55	Powder	Above pH 5.5
3	Eudragit L 100	Powder	Above pH 6.0
4	Eudragit L 12.5	12.5 % organic solution	Above pH 6.0
5	Eudragit S 100	Powder	Above pH 7.0
6	Eudragit S 12.5	12.5 % organic solution	Above pH 7.0
7	Eudragit FS 30D	30 % aqueous dispersion	Above pH 7.0
8	PVAP	Powder	Above pH 5.0
9	Shellac	Dry flakes	Above pH 7.0
10	HPMCP-50 and 55	Powder	Above pH 5.5
11	HPMCAS	Powder	Above pH 6.0
12	CAT	Powder	Above pH 5.5
	B. Time-dependent polymers		
13	Eudragit RS 100	Granules	Sustained release
14	Eudragit RL 100	Granules	Sustained release
15	Eudragit RL 12.5	12.5 % organic solution	Sustained release
16	Eudragit NE 30 D	30 % aqueous dispersion	Sustained release

N.B: PVAP; Polyvinyl acetate phthalate, HPMCP; Hydroxypropyl methylcellulose phthalate, HPMCAS; Hydroxypropyl methylcellulose acetate succinate, CAT; Cellulose acetate trimelitate.
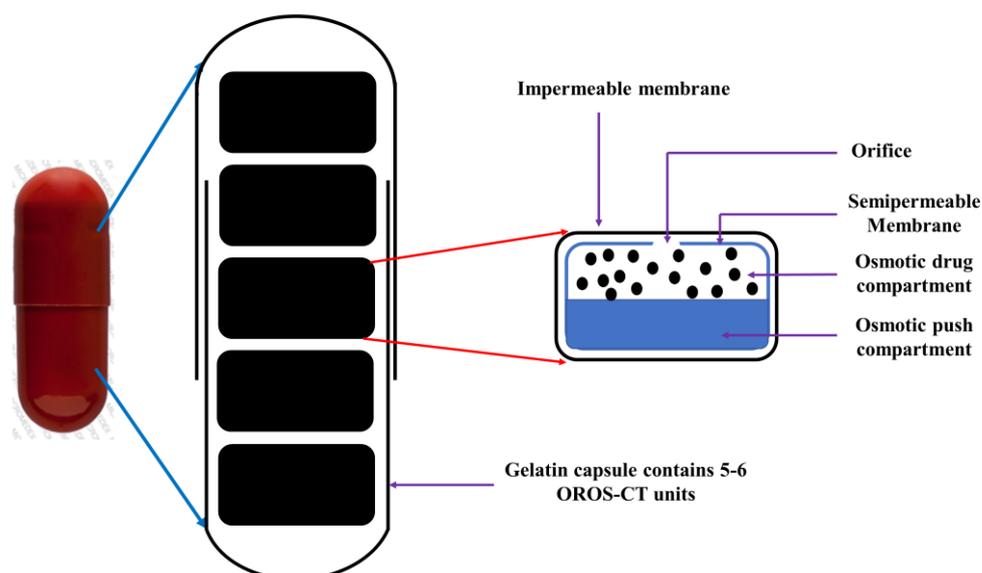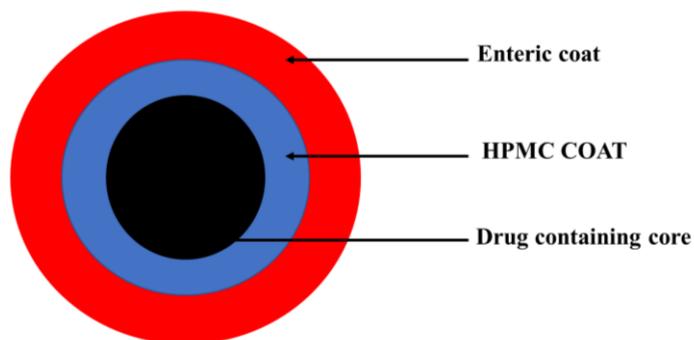

Figure 4: Schematic diagram of OROS-CT drug delivery system.
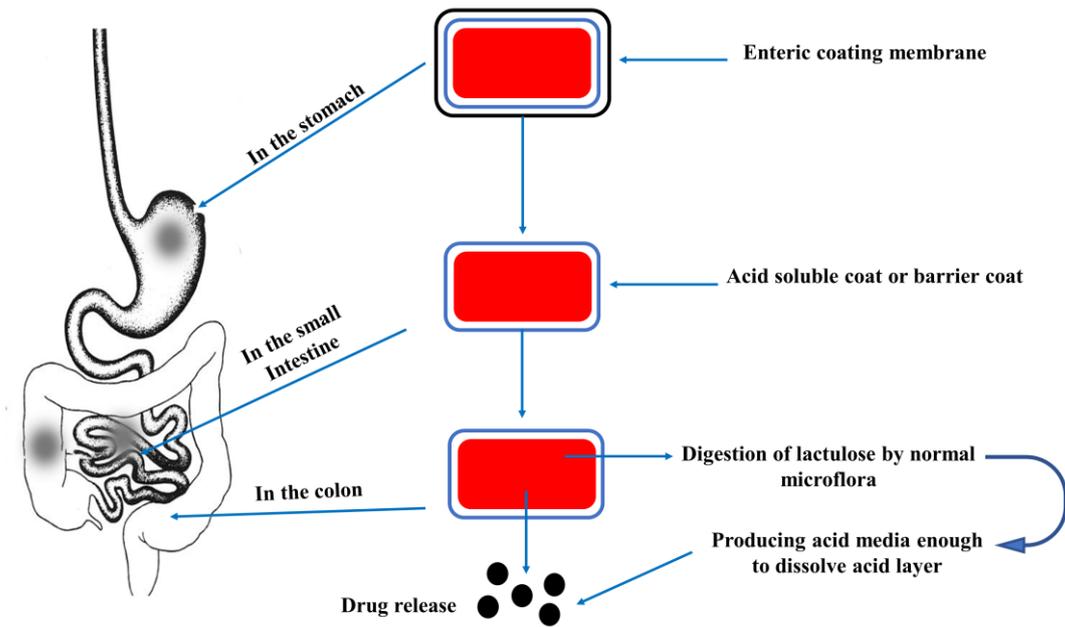
Figure 5: Schematic diagram of the new technology drug delivery system CODES^{TM}.
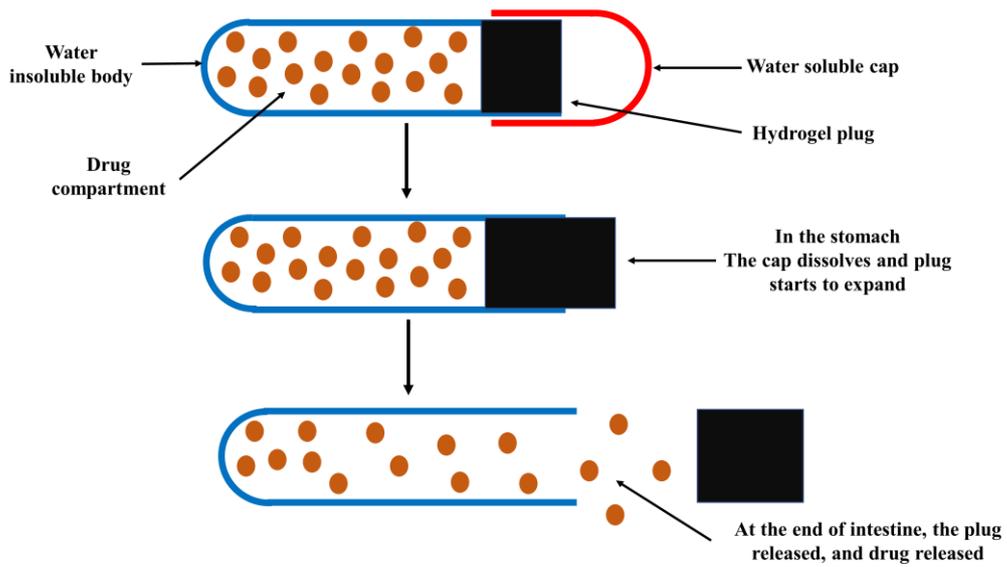
Figure 6: Schematic diagram of PulsinCap technology.

Figure 7: Schematic diagram of chronotropic drug delivery system.

Table 6: Advanced drug delivery systems for colon targeting.

No.	Drug carrier	Properties
1	COLAL® technology	Microflora activated system
2	MMX® technology	pH responsive system
3	PHLORAL® technology	pH and microflora activated system
4	Bioadhesive-based	Crosslinked polymers with charged coats
5	Redox based system	Azo reduction by enzymatically generated reduced flavins

5.6. Micro and Nano based drug delivery systems

5.6.1. Microparticles and IBD

In the last decade, the technology of drug delivery systems was directed into the approaches of decreasing particle size as the lower particle size of drug carries was capable of providing many advantages such as higher surface area, alteration of drug biodistribution and clearance, and the ability to target specific components in the inflammatory cascade such as in IBD. Coating drugs with biodegradable polymers in the size of microparticles providing a gastroprotective property and allowing the transportation of higher drug loading into the targeted site. Many studies showed an effective microparticles drug delivery systems for the treatment of IBD [118] **(Table 7)**.

5.6.2. Liposomes and IBD.

Liposomal drug delivery systems for the colon targeting could be used after the inclusion of gastroprotective polymeric coat at the surface of liposomes or by encapsulating liposomes inside gastro-resistant capsules. The polymeric coats will protect the liposomes from the hostile environment of the GIT and protect the bilayer lipid from the digestion by bile salts and digestive enzymes. Many polymeric coats could be manipulated for this purpose such as chitosan, Eudragit L 100, Eudragit S 100, and pectin [119].

5.6.3. Nanotechnology and IBD.

The term "nanotechnology" have many definitions as "the art of manipulating material on an atomic or molecular scale, especially to build microscopic devices" [120]. Also, defined as "the synthesis and the manipulation of particles having dimensions in nanometer scale" [121]. Another wide definition is "the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale" [122, 123].

From the point of medical view, a new term widely used related to nanotechnology is "nanobiotechnology" or "nanomedicine" or "nanomaterial" which is a branch of the science of drug delivery to specific cells in the form of nano-sized particles [124]. Nanomaterials having many advantages over microparticles or liposomes in drug delivery as the potential of nanomedicines to achieve both passive and active targeting to diseased location, ability to modify biodistribution and clearance of molecules, controlling drug release over time, and protection of drug molecules from degradation [125-127]. Nanoparticles for oral drug delivery are able to protect drug against environmental conditions of GIT, allow delivery of fragile drugs as proteins, peptides, and biological molecules as antibodies. More important, nanoparticles are able to passively target inflamed area, increase drug deposition at the diseased site, extended the desired pharmacological drug effect, and lower side effects. Based on that, nanoparticles have great potential to be a better drug delivery system for IBD [128-131]. Nanoparticles as drug delivery systems for the oral route having the ability to load and incorporate both hydrophilic and lipophilic drugs which allow ease of delivering both soluble and poorly soluble drugs.

Some physiological consideration to be taken into account to produce and design efficient colon targeted nanoparticles for the treatment of IBD. Transit time in patients with ulcerative colitis has a colonic transit time twice faster than normal persons due to high secretions and diarrhea, leading to challenges in targeting the colon using conventional formulations especially delayed systems [79]. Also, luminal pH changed during the active phase of inflammatory bowel disease [83]. Alterations in pH lead to change in the transit time and microbial flora contents which significantly affected drug release from traditional formulations [86]. In the same way, distortion of intestinal membrane integrity critically affected the drug deposition and absorption at the inflamed site [93-95]. Changes in intestinal contents, fluid volume, and microbial contents greatly affect nanoparticle activity.

From the previously discussed points, nanoparticles for IBD designed to overcome physiological conditions by its fundamental properties such as particle size and surface charge. Mean particle size and surface charge affect cellular uptake and interactions of nanoparticles with biomolecules. Generally, particles with size about 100 nm having more binding to the inflamed area compared to microparticles [132]. On the other hand, nanoparticles characterized by higher surface area-to-volume ratio have rapid drug release [133].

5.7. Nanotechnology strategies for drug delivery to IBD.

5.7.1. Nano-delivery of small molecules.

Nanoparticles for delivering small drug molecules designed in a manner which allowed efficient drug targeting. Many strategies used for nanoparticle preparation and surface decoration to allow drug deposition in the colon with efficient drug absorption and minimum undesirable effects.

The first strategy depends mainly on particle size reduction to the nanoscale range. Particles in nano-range showed many advantages over larger particles such as efficient colon transport and targeting through improving colonic residence time in the inflamed regions [134]. Also, smaller size allowed particles uptake by targeting immune cells like macrophages and decrease rapid elimination due to diarrhea which characterizes IBD [135]. This explained that accumulation of particles in the inflamed cells is size-dependent [132].

Another strategy is surface decorated nano-delivery systems, many techniques have been used to modify nanoparticles' surface to achieve good nanoparticle targeting, drug release retardation and increase drug distribution by preventing opsonization and mucus membrane adherence [136]. A study carried out by *Lautenschlager et al* [137] about the preparation of PEG-modified PLGA nanoparticles (300 nm) and microparticles (3000 nm). Modification of nanoparticles with PEG showed significantly enhanced particle translocation and deposition in the inflamed area compared to chitosan and non-coated PLGA nanoparticles. The PEG-modified surface is the most common and most applicable surface decoration mechanism [137, 138].

A surface charged nano-delivery systems is another approach. CD is characterized by excessive mucus secretion forming a thick mucus layer in the inflamed area. Mucus layer is composed mainly of mucins (a long chain hydrocarbons substrates with sulfates and sialic acid residues) that provides a negatively charged surface. Anionic mucus provides a mucoadhesion property which considered a promising colon targeting strategy with increased drug retention in the inflamed area [139-143]. Cationic nanoparticles adhere efficiently with the mucosal membrane [144]. From the other side, IBD is characterized by highly inflammable regions in which the inflamed cells have higher levels of cationic charge as well as infiltration of eosinophil cationic protein (ECP) and transferrin has been presented in higher concentrations in inflamed cells [145-150]. Anionic nanoparticles attach to inflamed cells via electrostatic interaction, but the main challenge is the drug delivery system must cross through thick mucus layer present in IBD [132].

The key feature of the pH-dependent strategy for colon drug delivery is the difference in pH in various sites of GIT and the use of pH-sensitive polymers [82, 151]. The selected polymers must be able to resist drug release in the upper gastrointestinal tract (lower pH-regions) (152). The most simple method is coating dosage form with pH-sensitive polymers [153] such as Eudragit®. Methacrylic acid copolymers (Eudragit S100) that dissolve at pH above 7, (Eudragit L100) that dissolves at pH above 6 and a special type (Eudragit FS 30D) that dissolves at pH above 6.5 [66, 154]. Many studies showed a significant reduction in the drug release in upper GIT [155-164].

5.7.2. Nano-delivery of biological molecules

Delivery of biological molecules using nanoparticles provided not only targeting but also afford protection against the upper gastrointestinal environment. Also, resolved issues of shorter half-life time of labile biological molecules in blood circulation [26]. Many biological molecules were approved for the treatment of IBD as, monoclonal antibodies infliximab, adalimumab and certolizumab, low molecular-weight heparin (LMWH), CD98-siRNA, TNF-α-siRNA, and the anti-inflammatory tripeptide Lys-Pro-Val (KPV) [26, 165].

6. Preparation of nanoparticles for the treatment of IBD.

6.1. Methods of nanoparticles preparation.

The term nanoparticles are defined as solid, colloidal particles in the nanoscale range. The term nanoparticles are a collective term which includes any polymeric nanoparticles but specifically, describe both Nanospheres and Nanocapsules [166-168]. One of the most fundamental characters of the nanoparticles is their size, which is generally taken to be in the range of 5-10 nm with an upper limit of 1000 nm, but the obtained size is generally around 100-500 nm [168, 169]. Nanospheres are known as a matrix particle in which the drug molecules may be dissolved, dispersed in the polymer matrix. On the other hand, Nanocapsules are defined as vesicular systems in which the drug molecules are confined in a cavity core consisting of a liquid lipid or water and surrounded by polymeric membrane coat [169, 170].

6.1.1. Dispersion of preformed polymers (One-step methods)

The most common technique for the preparation of nanoparticles mainly used to manufacture nanoparticles in one-step by the dispersion of preformed polymers. Many biodegradable and biocompatible polymers are used i.e. poly (D,L-Lactide-co-glycolic acid) (PLGA) [171, 172], poly (lactic acid) (PLA) [173], poly-epsilon-caprolactone (PCL) [174], poly (cyanoacrylate) (PCA) [175, 176] and methacrylate copolymers as Eudragit® [177-182].

Nanoprecipitation method is the most common and widely used method [183-186]. Simple, rapid, less energy-consuming, and timesaving. Nanoprecipitation is known as solvent displacement method or interfacial deposition method [187]. For the synthesis of nanoparticles, the method requires two main phases first, solvent phase (organic phase) consisting of solvent as acetone, polymer, surfactant, and drug. Oil is required in case of nanocapsules preparation. Secondly, the non-solvent phase (aqueous phase) consisting of water or buffer and stabilizer. The organic phase should be completely miscible with non-solvent phase [188]. The method is based mainly on spontaneous

emulsification of organic phase into the non-solvent phase (aqueous phase) [189]. The rapid diffusion of solvent phase into the aqueous phase leads to precipitation and formation of nanoparticles [190].

The emulsification techniques are widely applicable methods for the preparation of nanoparticles that mainly depends upon the formation of a nanoemulsion firstly before the nanoparticle formation [189]. The techniques include emulsification-diffusion, emulsification-coacervation, emulsification-evaporation, and double or multiple emulsification methods. Emulsification-diffusion is the most common method and widely used for lipophilic drugs. The method was described by *Leroux et al.* [191] for the preparation of nanospheres and by *Quintanar et al.* [192] for the preparation of polymeric nanocapsules. Generally, the technique consisted of three main phases, organic phase, aqueous phase and dilution or external phase [193]. In this case, the organic solvent should be partially miscible with the non-solvent phase.

Many solvents i.e. benzyl alcohol [191], propylene carbonate [193] and ethyl acetate [194] could be used. The resulting size is about 150-200 nm. The emulsion-diffusion method is considered as a modification of emulsion-evaporation technique [195, 196]. On the same way, emulsification-evaporation technique or emulsification-solvent evaporation technique is a technique based mainly on the formation of O/W emulsion and suitable for the preparation of nanoparticles for lipophilic drugs [197]. The method is usually depending on the preparation of nanoemulsion and followed by solvent evaporation leading to polymer precipitation as nanoparticles [198]. The main drawback of this method is the formation of multiple interfaces in organic and aqueous phases leading to the restriction of solvent diffusion [189]. Furthermore, the multiple-emulsification technique and the most common form, the double-emulsification method is a modified form of the emulsion-evaporation technique [199, 200], in which multiple emulsions to be formed before solvent evaporation. The method is used for encapsulation of both hydrophilic and lipophilic drug molecules by the formation of W/O/W [201, 202] and O/W/O emulsions, respectively [203, 204]. Finally, the emulsion-coacervation method is mainly used for the manufacturing of nanoparticles from natural polymers like gelatin and sodium alginate [189]. The method depends mainly on the formation of nanoemulsion then coacervation which results in polymer precipitation. The coacervation can be done by many methods such as dehydrating agents [205], electrolyte addition [206, 207] and temperature modification [208]. In order to stabilize the aqueous dispersion of the prepared nanoparticles, a cross-linking step is required by the using of the cross-linking agent or by changing the temperature or the pH [205-208].

The salting-out technique is based upon the formation of the emulsion by a solvent which is totally miscible with the aqueous phase [209]. After the emulsification of the polymer is formed, the salting-out agent is used at a high concentration of salts or sucrose. Magnesium chloride, sodium chloride, calcium chloride, and magnesium acetate are commonly used electrolytes [210-215].

6.1.2. Polymerization of monomers (two-step methods)

In this method, the drug could be encapsulated during the formation of polymers from starting monomers or by adsorption on the prepared nanoparticles [168, 216]. Three main techniques used for the polymerization of monomers are emulsion-polymerization method, mini-emulsion, and microemulsion polymerization method. Excess drug and surfactant used during the preparation of nanoparticles could be removed either by flow filtration techniques or by centrifugation. Many monomers used for the preparation of nanoparticles by polymerization methods [217, 218].

7. Physico-chemical characterization of prepared nanoparticles

7.1. Behavior of nanoparticles as drug delivery systems

Nanoparticles properties and characterization are based upon some physicochemical properties like particle size, surface charge and the particle morphology [189]. It is very important properties for the interactions between the nanoparticles and biological systems and control nanoparticles therapeutic activity and its toxicity. Many techniques used for determination of particle size and particle size distribution as photon correlation spectroscopy (PCS), atomic forced microscopy (AFM), electron microscopy (EM) and dynamic light scattering (DLS). The surface charge or zeta-potential is a very important parameter that determines the total surface charge and used to predict the stability of nanoparticle dispersion [219].

7.2. *In-vitro* drug release from loaded nanoparticles

7.2.1. Barriers affecting oral drug delivery

Oral drug delivery systems and especially delivery to the distal region of the GIT encountered many barriers like the harsh acidic environment of the stomach and intestine, gastric and bacterial enzymes, mucus layer especially thicker mucus layer in IBD, and tight junctions of the epithelium [139, 220]. The acidic environment of the GIT includes highly acidic pH of the stomach which ranged from 1.2 to 2.5 and the pH-value raised to 6.6-7.5 at the duodenum and the distal part of the intestine then pH drops again to 6.4 at the cecum which making the design of nanoparticles more difficult [221, 222]. Also, the mucus layer that becomes thicker in the case of IBD and rapid turnover of mucus leading to the rapid clearance of nanoparticles rather than the physical barrier [223-225].

7.2.2. *In vitro* drug modeling for nanoparticles.

In order to develop a successful drug delivery system to the colon, the drug release from loaded nanoparticles is one of the very important factors that control drug delivery designs. The Release rate from loaded nanoparticles especially nanocapsules depends on a great variety of factors including nanocapsules-related factors i.e. drug concentration, drug solubility and oil/water partitioning, Physico-chemical properties, molecular

weight and concentration of the polymer matrix, the oil nature, and the size of the prepared nanocapsules. Release media conditions-related factors i.e. medium pH, medium temperature, release enhancers, and contact time. The method of the preparation-related factors i.e. method of the drug incorporation which includes adsorption and other incorporation techniques [226].

For *in-vitro* drug release analysis, three main methods had been used namely, 1. Sample and separate (SS) and its modification such as ultracentrifugation, ultrafiltration and centrifugal ultrafiltration technique, 2. Continuous flow (CF), and 3. Dialysis membrane (DM) and its modification such as dialysis bag diffusion technique and reverse dialysis sac technique [26, 227].

In the case of sample and separate method, the nanoparticles introduced into the release media at a constant temperature and agitation rate. At different time intervals, samples were taken (supernatant, filtrate or nanoparticles) and measured analytically [179, 228-231]. The nanoparticle solution is separated from the release media with two main methods. The first method is to separate nanoparticles from the release media after sampling by the mean of ultracentrifugation, ultrafiltration or centrifugal ultrafiltration, and for larger nanoparticles might require only filtration using syringe filter 0.45 µm. Sample analysis was carried out by the using of supernatant, filtrate or destructive techniques for analysis of separated nanoparticles, then the release media replaced with fresh media [138, 232-234]. The second method for nanoparticle separation is the using of dialysis membrane with specific MWCO, but the drug can be equilibrated between the two-compartment and nanoparticles cannot cross the dialysis membrane [235]. For the colon targeted nanoparticles, to simulate the colon conditions, release studies were performed in different pH-values [236, 237].

8. Biopharmaceutical aspects

Different studies have been introduced to study nanoparticles' cytotoxicity as human exposure to nanomedicines is inevitable. The most important tests for cell viability studies are LDH (lactate dehydrogenase) which is normally released by the destroyed and damaged cells, the amount of LDH is directly proportional to the number of dead cells. On the other hand, MTT (methyl thiazolyl tetrazolium) test is used to differentiate between dead and live cells. MTT is a pale yellow dye converted into dark blue farmazan product only in the viable cells and could be determined spectrophotometrically [238, 239].

In order to understand IBD and especially disease pathogenesis, animal models have been used and particularly mouse models. Experimental colitis could be induced by many techniques include chemically induced colitis, bacterial-induced colitis, and genetically induced colitis. Transgenic (Tg) and gene knockout (KO) strains have been developed as genetically-induced models [240, 241]. The most common chemical-induced models are dextran sodium sulfate (DSS) model [242-245], oxazolone

model, TNBS model [246-249], and acetic acid model [250, 251].

Acetic acid-induced colitis was performed by many techniques including instillation of 3-6 % of acetic acid (2 mL) transrectally for 2 minutes in rats and animals were kept in a horizontal position to avoid leakage of the solution then the colon was rinsed with saline. In the case of mice, injection of 4-5 % v/v of acetic acid (1 mL) in 0.9 saline solution in the colon lumen approximately about 4 cm from the anus [250, 251]. Successful colitis model was evaluated by the clinical scoring system depending on some criteria i.e. animal activity, bloody stool, diarrhea, animal weight, and histopathological examination of the colon.

Clinical application of nanoparticles for the treatment of IBD in humans is limited due to human patients are more complex than the animal models. Passive targeting technique for the treatment of IBD may not be sufficient to obtain a therapeutic outcome. Therefore, active targeting techniques such as targeting cell receptors which extensively expressed in the case of inflammation and mucus targeting are a promising technique for colitis treatment with lower adverse effects and higher drug therapeutic concentration at the site of inflammation.

Many studies should be done to successfully translate the concept of active targeting from animal studies to human application. In order to translate animal studies into the clinic, many studies should explain some of the important points about nanoparticles i.e. the safety of administered nanoparticles following uptake, studies about the stability of nanoparticle structure through the GIT transit, and in-vitro/ex vivo stability. Finally, increased drug residence time at the site of inflammation should be optimized. From another point of view, the commercial point, the design of nanoparticles for drug delivery to the colon requires being simplified to allow efficient manufacturing at a large scale [22]. A study by Schmidt et al. [252] showed that the application of PLGA nanoparticles and microparticles on human patients for the first time provides passive targeting depending on their particle size alone could be applied to human.

Conclusion:

Site-specific drug delivery systems offer many advantages over other drug carriers especially in the oral route such as protection of the drug from the harsh environment of the gastrointestinal tract, loading high amount of the drug to the site of action, and decreasing unwanted side effects. Colon drug delivery systems are one of the most rapidly growing delivery technologies in the pharmaceutical field. The newly developed systems are directed to treat local diseases such as colon cancer, inflammatory bowel disease, and other colon conditions. Also, many colon drug delivery systems are used for the protection of drugs and biologically active ingredients such as peptides and antibodies which easily degraded in the upper gastrointestinal tract. All colon drug delivery systems even the newly developed technologies are based on three colon conditions: pH of the colon, transit time, and microbial content.

References

[1] Hirani JJ, Rathod DA, Vadalia KR. Orally disintegrating tablets: *a review*. *Trop J Pharm Res*. 2009;8(2):161-72.

[2] Coelho JF, Ferreira PC, Alves P, Cordeiro R, Fonseca AC, Góis JR, et al. Drug delivery systems: Advanced technologies potentially applicable in personalized treatments. *The EPMA Journal*. 2010;1(1):164-209.

[3] Yadav V, Gaisford S, Merchant HA, Basit AW. Colonic bacterial metabolism of corticosteroids. *International journal of pharmaceutics*. 2013;457(1):268-74.

[4] Chien YW, Swarbrick J. Novel drug delivery systems. 1992.

[5] Chourasia M, Jain S. Pharmaceutical approaches to colon targeted drug delivery systems. *J Pharm Pharm Sci*. 2003;6(1):33-66.

[6] Ohara T, Kitamura S, Kitagawa T, Terada K. Dissolution mechanism of poorly water-soluble drug from extended release solid dispersion system with ethylcellulose and hydroxypropylmethylcellulose. *International journal of pharmaceutics*. 2005;302(1):95-102.

[7] Krishnaiah Y, Satyanarayana S, Prasad YR, Rao SN. Evaluation of guar gum as a compression coat for drug targeting to colon. *International journal of pharmaceutics*. 1998;171(2):137-46.

[8] Rathbone MJ. Controlled Release in Oral Drug Delivery. Rathbone MJ, editor. New York Dordrecht Heidelberg London: *springer*; 2013 2011. 415 p.

[9] Basit AW, McConnell EL. Drug Delivery to the Colon. In: Wilson CG, Crowley PJ, editors. *Controlled Release in Oral Drug Delivery. Boston, MA: Springer US;* 2011. p. 385-99.

[10] Philip AK, Philip B. Colon targeted drug delivery systems: a review on primary and novel approaches. *Oman medical journal*. 2010;25(2):79-87.

[11] Friend DR. Colon-specific drug delivery. *Advanced drug delivery reviews*. 1991;7(1):149-99.

[12] Tortora GJ, Derrickson BH. Principles of anatomy and physiology: *John Wiley & Sons*; 2008.

[13] Meyers M. The Colon: Normal and Pathologic Anatomy. Dynamic Radiology of the Abdomen: Normal and Pathologic Anatomy. *New York, NY: Springer New York;* 2005. p. 665-709.

[14] Rajilić-Stojanović M, Smidt H, De Vos WM. Diversity of the human gastrointestinal tract microbiota revisited. *Environmental microbiology*. 2007;9(9):2125-36.

[15] Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. *International journal of pharmaceutics*. 2008;363(1):1-25.

[16] Wilding I. The enterion capsule: a novel technology for understanding the biopharmaceutical complexity of new molecular entities (NMEs). *Drug Deliv Tech*. 2001;1(1):8-11.

[17] Tuleu C, Basit A, Waddington W, Ell P, Newton J. Colonic delivery of 4-aminosalicylic acid using amylose–ethylcellulose-coated hydroxypropylmethylcellulose capsules. *Alimentary pharmacology & therapeutics*. 2002;16(10):1771-9.

[18] Coco R, Plapied L, Pourcelle V, Jerome C, Brayden DJ, Schneider YJ, et al. Drug delivery to inflamed colon by nanoparticles: comparison of different strategies. *International journal of pharmaceutics*. 2013;440(1):3-12.

[19] Carter MJ, Lobo AJ, Travis SP. Guidelines for the management of inflammatory bowel disease in adults. *Gut*. 2004;53(suppl 5):v1-v16.

[20] Peyrin-Biroulet L, Sandborn W, Sands B, Reinisch W, Bemelman W, Bryant R, et al. Selecting therapeutic targets in inflammatory bowel disease (STRIDE): determining therapeutic goals for treat-to-target. *The American journal of gastroenterology*. 2015;110(9):1324-38.

[21] Hanauer SB, Robinson M, Pruitt R, Lazenby AJ, Persson T, Nilsson LG, et al. Budesonide enema for the treatment of active, distal ulcerative colitis and proctitis: A dose-ranging study. *Gastroenterology*. 1998;115(3):525-32.

[22] Hua S, Marks E, Schneider JJ, Keely S. Advances in oral nano-delivery systems for colon targeted drug delivery in inflammatory bowel disease: selective targeting to diseased versus healthy tissue. *Nanomedicine : nanotechnology, biology, and medicine*. 2015;11(5):1117-32.

[23] Walker BR, Colledge NR. Davidson's principles and practice of medicine: *Elsevier Health Sciences*; 2013.

[24] Fauci AS. Harrison's principles of internal medicine: *McGraw-Hill, Medical Publishing Division*; 2008.

[25] Klotz U, Schwab M. Topical delivery of therapeutic agents in the treatment of inflammatory bowel disease. *Advanced Drug Delivery Reviews*. 2005;57(2):267-79.

[26] Ali H, Collnot E-M, Windbergs M, Lehr C-M. Nanomedicines for the treatment of inflammatory bowel diseases. *European Journal of Nanomedicine*. 2013;5(1):23-38.

[27] Podolsky DK. Inflammatory bowel disease. *New England Journal of Medicine*. 1991;325(14):1008-16.

[28] Meissner Y, Lamprecht A. Alternative drug delivery approaches for the therapy of inflammatory bowel disease. *Journal of pharmaceutical sciences*. 2008;97(8):2878-91.

[29] Han J, Wang J, Wang JH. How to achieve deep remission in the treatment of inflammatory bowel disease. *Journal of Traditional Chinese Medicine*. 2013;33(4):549-52.

[30] Fiorino G, Fries W, De La Rue S, Malesci A, Repici A, Danese S. New drug delivery systems in inflammatory bowel disease: MMX™ and tailored delivery to the gut. *Current medicinal chemistry*. 2010;17(17):1851-7.

[31] Kaplan GG, Ng SC. Understanding and Preventing the Global Increase of Inflammatory Bowel Disease. *Gastroenterology*. 2017;152(2):313-21. e2.

[32] Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. *Gastroenterology*. 2017;152(2):327-39. e4.

[33] Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*. 2001;411(6837):603-6.

[34] Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Núñez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB. *Journal of Biological Chemistry*. 2001;276(7):4812-8.

[35] Horowitz J, Warner N, Staples J, Crowley E, Murchie R, Van Hout C, et al. Mutation spectrum of NOD2 reveals recessive inheritance as a main driver of Early Onset Crohn′s Disease. *bioRxiv*. 2017;098574.

[36] Koutroubakis I, Manousos O, Meuwissen S, Pena A. Environmental risk factors in inflammatory bowel disease. *Hepato-gastroenterology*. 1995;43(8):381-93.

[37] Ananthakrishnan AN. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol*. 2013;9(6):367-74.

[38] Molodecky NA, Kaplan GG. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol (NY)*. 2010;6(5):339-46.

[39] Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World Journal of Gastroenterology*. 2006;12(38):6102-8.

[40] Irvine EJ, Marshall JK. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. *Gastroenterology*. 2000;119(6):1740-4.

[41] Söderholm JD, Olaison G, Peterson K, Franzen L, Lindmark T, Wirén M, et al. Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's disease. *Gut*. 2002;50(3):307-13.

[42] Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology*. 2005;129(2):550-64.

[43] Pallone F, Monteleone G. Interleukin 12 and Th1 responses in inflammatory bowel disease. *Gut*. 1998;43(6):735-6.

[44] Boirivant M, Marini M, Di Felice G, Pronio AM, Montesani C, Tersigni R, et al. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology*. 1999;116(3):557-65.

[45] Modigliani R, Mary J-Y, Simon J-F, Cortot A, Soule J-C, Gendre J-P, et al. Clinical, biological, and endoscopic picture of attacks of Crohn's disease. Evolution on prednisolone. Groupe d'Etude Thérapeutique des Affections Inflammatoires Digestives. *Gastroenterology*. 1990;98(4):811-8.

[46] Gisbert JP, Gomollón F, Maté J, Pajares JM. REVIEW: Role of 5-Aminosalicylic Acid (5-ASA) in Treatment of Inflammatory Bowel Disease: A Systemic Review. *Digestive diseases and sciences*. 2002;47(3):471-88.

[47] Criscuoli V, Modesto I, Orlando A, Cottone M. Mesalazine for the treatment of inflammatory bowel disease. *Expert opinion on pharmacotherapy*. 2013;14(12):1669-78.

[48] Meyers S, Sachar DB, Present DH, Janowitz HD. Olsalazine sodium in the treatment of ulcerative colitis among patients intolerant of sulfasalazine: A prospective, randomized placebo-controlled, double-blind, dose-ranging clinical trial. *Gastroenterology*. 1987;93(6):1255-62.

[49] Grevenitis P, Thomas A, Lodhia N. Medical Therapy for Inflammatory Bowel Disease. *Surgical Clinics of North America*. 2015;95(6):1159-82.

[50] Pruitt R, Hanson J, Safdi M, Wruble L, Hardi R, Johanson J, et al. Balsalazide is superior to mesalamine in the time to improvement of signs and symptoms of acute mild-to-moderate ulcerative colitis. *The American journal of gastroenterology*. 2002;97(12):3078-86.

[51] Green JR, Lobo AJ, Holdsworth CD, Leicester RJ, Gibson JA, Kerr GD, et al. Balsalazide is more effective and better tolerated than mesalamine in the treatment of acute ulcerative colitis. *Gastroenterology*. 1998;114(1):15-22.

[52] Winship DH, Summers RW, Singleton JW, Best WR, Becktel JM, Lenk LF, et al. National Cooperative Crohn's Disease Study: study design and conduct of the study. *Gastroenterology*. 1979;77(4 Pt 2):829-42.

[53] Faubion WA, Loftus EV, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology*. 2001;121(2):255-60.

[54] McPhee SJ, Papadakis MA, Tierney LM. Current medical diagnosis & treatment 2010: *McGraw-Hill Medical New York*:; 2010.

[55] Van Assche G, Manguso F, Zibellini M, Nuño JLC, Goldis A, Tkachenko E, et al. Oral prolonged release beclomethasone dipropionate and prednisone in the treatment of active ulcerative colitis: results from a double-blind, randomized, parallel group study. *The American journal of gastroenterology*. 2015;110(5):708-15.

[56] Bar–Meir S, Chowers Y, Lavy A, Abramovitch D, Sternberg A, Leichtmann G, et al. Budesonide versus prednisone in the treatment of active Crohn's disease. *Gastroenterology*. 1998;115(4):835-40.

[57] Kolkman JJ, Möllman HW, Möllman AC, Nelis FG, Viergever P, Peñna S. Beneficial effect of oral budesonide for distal ulcerative colitis: A comparative study of Budenofalk® 3 mg tid vs 9 MG OD. *Gastroenterology*. 2000;118(4, Part 1):A779.

[58] HO GT, Chiam P, Drummond H, Loane J, Arnott I, Satsangi J. The efficacy of corticosteroid therapy in inflammatory bowel disease: analysis of a 5-year UK inception cohort. *Alimentary pharmacology & therapeutics*. 2006;24(2):319-30.

[59] Fraser A, Orchard T, Jewell D. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. *Gut*. 2002;50(4):485-9.

[60] Present DH, Meltzer SJ, Krumholz MP, Wolke A, Korelitz BI. 6-Mercaptopurine in the management of inflammatory bowel disease: short-and long-term toxicity. *Annals of Internal Medicine*. 1989;111(8):641-9.

[61] Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *The Lancet*. 2007;369(9573):1641-57.

[62] Robert C, Kupper TS. Inflammatory skin diseases, T cells, and immune surveillance. *New England Journal of Medicine*. 1999;341(24):1817-28.

[63] Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M, et al. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Molecular immunology*. 1993;30(16):1443-53.

[64] Swora E, Samborski P, Raniszewska M. Biological treatment of inflammatory bowel disease. *Nowiny Lek*. 2009;78:3-4.

[65] Yang L, Chu JS, Fix JA. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. *International journal of pharmaceutics*. 2002;235(1):1-15.

[66] Asghar LFA, Chandran S. Multiparticulate formulation approach to colon specific drug delivery: current perspectives. *J Pharm Pharm Sci*. 2006;9(3):327-38.

[67] Vyas S, Roop K. Khar (ed). Systems for colon specific drug delivery. Controlled drug delivery concepts and advances, 1st ed, *Delhi*. 2006:218-56.

[68] Sarasija S, Hota A. Colon-specific drug delivery systems. *Indian journal of pharmaceutical sciences*. 2000;62(1):1-8.

[69] Verma S, Kumar V, Mishra D, Singh S. Colon targeted drug delivery: current and novel perspectives. International Journal of Pharmaceutical Sciences and Research. 2012;3(5):1274.

[70] Kinget R, Kalala W, Vervoort L, Van den Mooter G. Colonic drug targeting. Journal of drug targeting. 1998;6(2):129-49.

[71] Nugent S, Kumar D, Rampton D, Evans D. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. Gut. 2001;48(4):571-7.

[72] Kolte BP. Colon targeted drug delivery system-a novel perspective. Asian journal of biomedical and pharmaceutical sciences. 2012;2(14):21.

[73] Arora S, Ali J, Ahuja A, Baboota S, Qureshi J. Pulsatile drug delivery systems: An approach for controlled drug delivery. Indian Journal of Pharmaceutical Sciences. 2006.

[74] Aggarwal S, Sharma S, Lal S, Choudhary N. Recent trend in colon targeted drug delivery system. RJPBS. 2011;2:406-15.

[75] Ahrabi SF, Madsen G, Dyrstad K, Sande SA, Graffner C. Development of pectin matrix tablets for colonic delivery of model drug ropivacaine. European Journal of Pharmaceutical Sciences. 2000;10(1):43-52.

[76] Vyas S, Khar R. Gastro-retentive system In: Controlled Drug Delivery System: Concept & Advances. Ist Ed New Delhi: Vallabh Prakashan. 2002;1(2):417-41.

[77] Wood E, Wilson CG, Hardy JG. The spreading of foam and solution enemas. International journal of pharmaceutics. 1985;25(2):191-7.

[78] Rana S, Sharma S, Malik A, Kaur J, Prasad K, Sinha S, et al. Small intestinal bacterial overgrowth and orocecal transit time in patients of inflammatory bowel disease. Digestive diseases and sciences. 2013;58(9):2594-8.

[79] Hebden Ja, Blackshaw P, Perkins A, Wilson C, Spiller R. Limited exposure of the healthy distal colon to orally-dosed formulation is further exaggerated in active left-sided ulcerative colitis. Alimentary Pharmacology and Therapeutics. 2000;14(2):155-62.

[80] Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology. 2008;134(2):577-94.

[81] Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. Gut. 2004;53(1):1-4.

[82] Fallingborg J, Christensen LA, Jacobsen BA, Rasmussen SN. Very low intraluminal colonic pH in patients with active ulcerative colitis. Digestive diseases and sciences. 1993;38(11):1989-93.

[83] Sasaki Y, Hada R, Nakajima H, Fukuda S, Munakata A. Improved localizing method of radiopill in measurement of entire gastrointestinal pH profiles: colonic luminal pH in normal subjects and patients with Crohn's disease. American Journal of Gastroenterology. 1997;92(1).

[84] Yuasa H. Drug absorption from the colon in situ. Drug absorption studies: Springer; 2008. p. 77-88.

[85] Ibekwe VC, Fadda HM, McConnell EL, Khela MK, Evans DF, Basit AW. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. Pharmaceutical research. 2008;25(8):1828-35.

[86] McConnell EL, Fadda HM, Basit AW. Gut instincts: explorations in intestinal physiology and drug delivery. International journal of pharmaceutics. 2008;364(2):213-26.

[87] Roda G, Sartini A, Zambon E, Calafiore A, Marocchi M, Caponi A, et al. Intestinal epithelial cells in inflammatory bowel diseases. *World Journal of Gastroenterology : WJG*. 2010;16(34):4264-71.

[88] Farhadi A, Banan A, Fields J, Keshavarzian A. Intestinal barrier: an interface between health and disease. *Journal of gastroenterology and hepatology*. 2003;18(5):479-97.

[89] Groschwitz KR, Hogan SP. Intestinal Barrier Function: Molecular Regulation and Disease Pathogenesis. *The Journal of allergy and clinical immunology*. 2009;124(1):3-22.

[90] Michielan A, D'Incà R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. *Mediators of Inflammation*. 2015;2015:628157.

[91] Goggins BJ, Chaney C, Radford-Smith GL, Horvat JC, Keely S. Hypoxia and integrin-mediated epithelial restitution during mucosal inflammation. *Frontiers in immunology*. 2013;4.

[92] Söderholm JD, Peterson KH, Olaison G, Franzén LE, Weström B, Magnusson K-E, et al. Epithelial permeability to proteins in the noninflamed ileum of Crohn's disease? *Gastroenterology*. 1999;117(1):65-72.

[93] Wang X, Maher S, Brayden DJ. Restoration of rat colonic epithelium after in situ intestinal instillation of the absorption promoter, sodium caprate. *Therapeutic delivery*. 2010;1(1):75-82.

[94] Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. *Cancer research*. 2002;62(12):3387-94.

[95] Creed T, Probert C. Review article: steroid resistance in inflammatory bowel disease–mechanisms and therapeutic strategies. *Alimentary pharmacology & therapeutics*. 2007;25(2):111-22.

[96] Carrette O, Favier C, Mizon C, Neut C, Cortot A, Colombel J, et al. Bacterial enzymes used for colon-specific drug delivery are decreased in active Crohn's disease. *Digestive diseases and sciences*. 1995;40(12):2641-6.

[97] Carrette O, Favier C, Mizon C, Neut C, Cortot A, Colombel JF, et al. Bacterial enzymes used for colon-specific drug delivery are decreased in active Crohn's disease. *Digestive Diseases and Sciences*. 1995;40(12):2641-6.

[98] Khan AA, Piris J, Truelove S. An experiment to determine the active therapeutic moiety of sulphasalazine. *The Lancet*. 1977;310(8044):892-5.

[99] Campieri M. New steroids and new salicylates in inflammatory bowel disease: a critical appraisal. *Gut*. 2002;50(suppl 3):iii43-iii6.

[100] Gangurde HH, Chordiya MA, Tamizharasi S, Sivakumar T. Diseases, approaches and evaluation parameterrs for colon specific drug delivery: a review. *International Journal of Drug Research and Technology*. 2017;2(3):23.

[101] Singh A, Sharma A. Novel approaches for colon targeted drug delivery system. 2014.

[102] Singh KI, Singh J, Sharma D, Sharma A. Colon specific drug delivery system: review on novel approaches. *International Journal of Pharmaceutical Sciences and Research*. 2012;3(3):637.

[103] Jose S, Dhanya K, Cinu TA, Litty J, Chacko AJ. Colon targeted drug delivery: Different approaches. *Journal of Young Pharmacists*. 2009;1(1):13.

[104] Lautenschläger C, Schmidt C, Fischer D, Stallmach A. Drug delivery strategies in the therapy of inflammatory bowel disease. *Advanced Drug Delivery Reviews*. 2014;71:58-76.

[105] Aurora J, Talwar N, Pathak V. Colonic drug delivery challenges and opportunities-an overview. *European Gastroenterology Review*. 2006;1:1-4.

[106] Antonin K, Rak R, Bieck P, Preiss R, Schenker U, Hastewell J, et al. The absorption of human calcitonin from the transverse colon of man. *International journal of pharmaceutics*. 1996;130(1):33-9.

[107] Anuj S, Amit JK. Colon targeted drug delivery using different approaches. *Int J Pharm Stud Res*. 2010;1(1):60.

[108] Basit AW. Advances in colonic drug delivery. *Drugs*. 2005;65(14):1991-2007.

[109] Davis S, Hardy J, Fara J. Transit of pharmaceutical dosage forms through the small intestine. *Gut*. 1986;27(8):886-92.

[110] Davis S, Hardy J, Taylor M, Whalley D, Wilson C. The effect of food on the gastrointestinal transit of pellets and an osmotic device (Osmet). *International journal of pharmaceutics*. 1984;21(3):331-40.

[111] Devereux J, Newton J, Short M. The influence of density on the gastrointestinal transit of pellets. *Journal of Pharmacy and Pharmacology*. 1990;42(7):500-1.

[112] Colombo P, Bettini R, Massimo G, Catellani PL, Santi P, Peppas NA. Drug diffusion front movement is important in drug release control from swellable matrix tablets. *Journal of pharmaceutical sciences*. 1995;84(8):991-7.

[113] Maroni A, Zema L, Cerea M, Foppoli A, Palugan L, Gazzaniga A. Erodible drug delivery systems for time-controlled release into the gastrointestinal tract. *Journal of Drug Delivery Science and Technology*. 2016;32, Part B:229-35.

[114] Takaya T, Niwa K, Muraoka M, Ogita I, Nagai N, Yano R-i, et al. Importance of dissolution process on systemic availability of drugs delivered by colon delivery system. *Journal of Controlled Release*. 1998;50(1):111-22.

[115] Prasanth V, Jayaprakash R, Mathew ST. Colon Specific Drug Delivery Systems: *A Review on Various Pharmaceutical Approaches*. 2012.

[116] Amidon S, Brown JE, Dave VS. Colon-Targeted Oral Drug Delivery Systems: Design Trends and Approaches. *AAPS PharmSciTech*. 2015;16(4):731-41.

[117] Choudhury PK, Panigrahi TK, Murthy PN, Tripathy NK, Behera S, Panigrahi R. Novel approaches and developments in colon specific drug delivery systems-a review. 2012.

[118] Nidhi, Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. Saudi pharmaceutical journal : *SPJ : the official publication of the Saudi Pharmaceutical Society*. 2016;24(4):458-72.

[119] Gupta AS, Kshirsagar SJ, Bhalekar MR, Saldanha T. Design and development of liposomes for colon targeted drug delivery. *Journal of Drug Targeting*. 2013;21(2):146-60.

[120] Dictionary M-W. Merriam-Webster Online Dictionary. *Retrieved November*. 2010;6.

[121] Viscido A, Capannolo A, Latella G, Caprilli R, Frieri G. Nanotechnology in the treatment of inflammatory bowel diseases. *Journal of Crohn's and Colitis*. 2014;8(9):903-18.

[122] Emerich DF, Thanos CG. Nanotechnology and medicine. *Expert Opinion on Biological Therapy*. 2003;3(4):655-63.

[123] Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. *Drug discovery today*. 2003;8(24):1112-20.

[124] Maitra A. Opinion: Does nanomedicine really belong to the field of nanotechnology. 2010.

[125] Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacological reviews*. 2001;53(2):283-318.

[126] Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Advanced Drug Delivery Reviews*. 2003;55(3):329-47.

[127] Singh R, Lillard Jr JW. Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology*. 2009;86(3):215-23.

[128] Ulbrich W, Lamprecht A. Targeted drug-delivery approaches by nanoparticulate carriers in the therapy of inflammatory diseases. *Journal of The Royal Society Interface*. 2009:rsif20090285.

[129] Mahajan N, Sakarkar D, Manmode A, Pathak V, Ingole R, Dewade D. Biodegradable nanoparticles for targeted delivery in treatment of ulcerative colitis. *Advanced Science Letters*. 2011;4(2):349-56.

[130] Xiao B, Merlin D. Oral colon-specific therapeutic approaches toward treatment of inflammatory bowel disease. *Expert opinion on drug delivery*. 2012;9(11):1393-407.

[131] Collnot E-M, Ali H, Lehr C-M. Nano-and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. *Journal of Controlled Release*. 2012;161(2):235-46.

[132] Lamprecht A, Schäfer U, Lehr C-M. Size-dependent bioadhesion of micro-and nanoparticulate carriers to the inflamed colonic mucosa. *Pharmaceutical research*. 2001;18(6):788-93.

[133] Hörter D, Dressman J. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Advanced drug delivery reviews*. 2001;46(1):75-87.

[134] Hua S, Marks E, Schneider JJ, Keely S. Advances in oral nano-delivery systems for colon targeted drug delivery in inflammatory bowel disease: Selective targeting to diseased versus healthy tissue. Nanomedicine: *Nanotechnology, Biology and Medicine*. 2015;11(5):1117-32.

[135] Lamprecht A, Yamamoto H, Ubrich N, Takeuchi H, Maincent P, Kawashima Y. FK506 microparticles mitigate experimental colitis with minor renal calcineurin suppression. *Pharmaceutical research*. 2005;22(2):193-9.

[136] Owens Iii DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics*. 2006;307(1):93-102.

[137] Lautenschläger C, Schmidt C, Lehr C-M, Fischer D, Stallmach A. PEG-functionalized microparticles selectively target inflamed mucosa in inflammatory bowel disease. *European Journal of Pharmaceutics and Biopharmaceutics*. 2013;85(3):578-86.

[138] Danhier F, Lecouturier N, Vroman B, Jérôme C, Marchand-Brynaert J, Feron O, et al. Paclitaxel-loaded PEGylated PLGA-based nanoparticles: in vitro and in vivo evaluation. *Journal of Controlled Release*. 2009;133(1):11-7.

[139] Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. *World J Gastroenterol*. 2014;20(5):1165-79.

[140] Condello M, De Berardis B, Ammendolia MG, Barone F, Condello G, Degan P, et al. ZnO nanoparticle tracking from uptake to genotoxic damage in human colon carcinoma cells. *Toxicology in Vitro*. 2016;35:169-79.

[141] Larsson JMH, Karlsson H, Sjövall H, Hansson GC. A complex, but uniform O-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MSn. *Glycobiology*. 2009;19(7):756-66.

[142] Maisel K, Ensign L, Reddy M, Cone R, Hanes J. Effect of surface chemistry on nanoparticle interaction with gastrointestinal mucus and distribution in the gastrointestinal tract following oral and rectal administration in the mouse. *Journal of Controlled Release*. 2015;197:48-57.

[143] Salatin S, Maleki Dizaj S, Yari Khosroushahi A. Effect of the surface modification, size, and shape on cellular uptake of nanoparticles. *Cell biology international*. 2015;39(8):881-90.

[144] Niebel W, Walkenbach K, Béduneau A, Pellequer Y, Lamprecht A. Nanoparticle-based clodronate delivery mitigates murine experimental colitis. *Journal of controlled release*. 2012;160(3):659-65.

[145] Jubeh T, Barenholz Y, Rubinstein A. Differential adhesion of normal and inflamed rat colonic mucosa by charged liposomes. *Pharmaceutical research*. 2004;21(3):447-53.

[146] Bischoff S, Wedemeyer J, Herrmann A, Meier P, Trautwein C, Cetin Y, et al. Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. *Histopathology*. 1996;28(1):1-13.

[147] Carlson M, Raab Y, Peterson C, Hällgren R, Venge P. Increased intraluminal release of eosinophil granule proteins EPO, ECP, EPX, and cytokines in ulcerative colitis and proctitis in segmental perfusion. *The American journal of gastroenterology*. 1999;94(7):1876-83.

[148] Nagashima R. Mechanisms of action of sucralfate. *Journal of clinical gastroenterology*. 1980;3(Suppl 2):117-27.

[149] Peterson CG, Eklund E, Taha Y, Raab Y, Carlson M. A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease. *The American journal of gastroenterology*. 2002;97(7):1755-62.

[150] Tirosh B, Khatib N, Barenholz Y, Nissan A, Rubinstein A. Transferrin as a luminal target for negatively charged liposomes in the inflamed colonic mucosa. *Molecular pharmaceutics*. 2009;6(4):1083-91.

[151] Bratten J, Jones MP. New directions in the assessment of gastric function: clinical applications of physiologic measurements. *Digestive Diseases*. 2006;24(3-4):252-9.

[152] Davaran S, Rashidi M, Hashemi M. Synthesis and characterization of methacrylic derivatives of 5-amino salicylic acid with pH-sensitive swelling properties. *AAPS PharmSciTech*. 2001;2(4):80-5.

[153] Ashford M, Fell JT, Attwood D, Sharma H, Woodhead PJ. An in vivo investigation into the suitability of pH dependent polymers for colonic targeting. International journal of pharmaceutics. 1993;95(1-3):193-9.

[154] Joshi M. Role of Eudragit in targeted drug delivery. *Int J Curr Pharm Res*. 2013;5(2):58-62.

[155] Makhlof A, Tozuka Y, Takeuchi H. pH-Sensitive nanospheres for colon-specific drug delivery in experimentally induced colitis rat model. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009;72(1):1-8.

[156] Barea M, Jenkins M, Gaber M, Bridson R. Evaluation of liposomes coated with a pH responsive polymer. *International journal of pharmaceutics*. 2010;402(1):89-94.

[157] Kshirsagar SJ, Bhalekar MR, Patel JN, Mohapatra SK, Shewale NS. Preparation and characterization of nanocapsules for colon-targeted drug delivery system. *Pharmaceutical development and technology*. 2012;17(5):607-13.

[158] Ali H, Weigmann B, Collnot EM, Khan SA, Windbergs M, Lehr CM. Budesonide Loaded PLGA Nanoparticles for Targeting the Inflamed Intestinal Mucosa--Pharmaceutical Characterization and Fluorescence Imaging. *Pharmaceutical research*. 2016;33(5):1085-92.

[159] Ali H, Weigmann B, Neurath M, Collnot E, Windbergs M, Lehr C-M. Budesonide loaded nanoparticles with pH-sensitive coating for improved mucosal targeting in mouse models of inflammatory bowel diseases. *Journal of Controlled Release*. 2014;183:167-77.

[160] Naeem M, Choi M, Cao J, Lee Y, Ikram M, Yoon S, et al. Colon-targeted delivery of budesonide using dual pH-and time-dependent polymeric nanoparticles for colitis therapy. *Drug design, development and therapy*. 2015;9:3789.

[161] Naeem M, Kim W, Cao J, Jung Y, Yoo J-W. Enzyme/pH dual sensitive polymeric nanoparticles for targeted drug delivery to the inflamed colon. Colloids and Surfaces B: *Biointerfaces*. 2014;123:271-8.

[162] Beloqui A, Coco R, Memvanga PB, Ucakar B, des Rieux A, Préat V. pH-sensitive nanoparticles for colonic delivery of curcumin in inflammatory bowel disease. *International journal of pharmaceutics*. 2014;473(1):203-12.

[163] Lamprecht A, Yamamoto H, Takeuchi H, Kawashima Y. A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles. *Journal of Controlled release*. 2005;104(2):337-46.

[164] Qelliny MR, Aly UF, Elgarhy OH, Khaled KA. Budesonide-Loaded Eudragit S 100 Nanocapsules for the Treatment of Acetic Acid-Induced Colitis in Animal Model. *J AAPS PharmSciTech*. 2019;20(6):237.

[165] Rutgeerts P, Vermeire S, Van Assche G. Biological Therapies for Inflammatory Bowel Diseases. *Gastroenterology*. 2009;136(4):1182-97.

[166] Couvreur P. Polyalkylcyanoacrylates as colloidal drug carriers. *Critical reviews in therapeutic drug carrier systems*. 1987;5(1):1-20.

[167] Fattal E, Vauthier C. Nanoparticles as drug delivery systems. *Encyclopedia of Pharmaceutical Technology*. 2002;2:811-33.

[168] Vauthier C, Bouchemal K. Methods for the preparation and manufacture of polymeric nanoparticles. *Pharmaceutical research*. 2009;26(5):1025-58.

[169] Quintanar-Guerrero D, Allémann E, Fessi H, Doelker E. Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. *Drug development and industrial pharmacy*. 1998;24(12):1113-28.

[170] Vauthier C, Couvreur P. Development of nanoparticles made of polysaccharides as novel drug carrier systems. *Handbook of pharmaceutical controlled release technology,* Marcel Dekker, New York. 2000:413-29.

[171] Alshamsan A. Nanoprecipitation is more efficient than emulsion solvent evaporation method to encapsulate cucurbitacin I in PLGA nanoparticles. *Saudi Pharmaceutical Journal*. 2014;22(3):219-22.

[172] Stecanella LA, Taveira SF, Marreto RN, Valadares MC, Vieira MdS, Kato MJ, et al. Development and characterization of PLGA nanocapsules of grandisin isolated from Virola surinamensis: in vitro release and cytotoxicity studies. *Revista Brasileira de Farmacognosia*. 2013;23(1):153-9.

[173] Pandey SK, Patel DK, Thakur R, Mishra DP, Maiti P, Haldar C. Anti-cancer evaluation of quercetin embedded PLA nanoparticles synthesized by emulsified nanoprecipitation. *International journal of biological macromolecules*. 2015;75:521-9.

[174] Byun Y, Hwang JB, Bang SH, Darby D, Cooksey K, Dawson PL, et al. Formulation and characterization of α-tocopherol loaded poly ε-caprolactone (PCL) nanoparticles. *LWT-Food Science and Technology*. 2011;44(1):24-8.

[175] González-Martín G, Figueroa C, Merino I, Osuna A. Allopurinol encapsulated in polycyanoacrylate nanoparticles as potential lysosomatropic carrier: preparation and trypanocidal activity. *European journal of pharmaceutics and biopharmaceutics*. 2000;49(2):137-42.

[176] Sham JO-H, Zhang Y, Finlay WH, Roa WH, Löbenberg R. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. *International Journal of Pharmaceutics*. 2004;269(2):457-67.

[177] Ahuja M, Dhake AS, Sharma SK, Majumdar DK. Diclofenac-loaded Eudragit S100 nanosuspension for ophthalmic delivery. *Journal of microencapsulation*. 2011;28(1):37-45.

[178] Cerchiara T, Bigucci F, Corace G, Zecchi V, Luppi B. Eudragit-coated albumin nanospheres carrying inclusion complexes for oral administration of indomethacin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*. 2011;71(1-2):129-36.

[179] Cetin M, Atila A, Kadioglu Y. Formulation and in vitro characterization of Eudragit® L100 and Eudragit® L100-PLGA nanoparticles containing diclofenac sodium. *AAPS PharmSciTech*. 2010;11(3):1250-6.

[180] Mandal B. Preparation and physicochemical characterization of Eudragit® RL100 Nanosuspension with potential for Ocular Delivery of Sulfacetamide: *University of Toledo*; 2010.

[181] Santos SS, Lorenzoni A, Ferreira LM, Mattiazzi J, Adams AI, Denardi LB, et al. Clotrimazole-loaded Eudragit® RS100 nanocapsules: Preparation, characterization and in vitro evaluation of antifungal activity against Candida species. *Materials Science and Engineering: C*. 2013;33(3):1389-94.

[182] Yadav SK, Mishra S, Mishra B. Eudragit-Based Nanosuspension of Poorly Water-Soluble Drug: Formulation and In Vitro–In Vivo Evaluation. *AAPS PharmSciTech*. 2012;13(4):1031-44.

[183] Aghajani M, Shahverdi AR, Rezayat SM, Amini MA, Amani A. Preparation and optimization of acetaminophen nanosuspension through nanoprecipitation using microfluidic devices: an artificial neural networks study. *Pharmaceutical development and technology*. 2013;18(3):609-18.

[184] Bilati U, Allémann E, Doelker E. Nanoprecipitation versus emulsion-based techniques for the encapsulation of proteins into biodegradable nanoparticles and process-related stability issues. *Aaps Pharmscitech*. 2005;6(4):E594-E604.

[185] Jayanta K. Critical process parameters evaluation of modified nanoprecipitation method on lomustine nanoparticles and cytostatic activity study on L132 human cancer cell line. *Journal of Nanomedicine & Nanotechnology*. 2012;3:8.

[186] Lepeltier E, Bourgaux C, Couvreur P. Nanoprecipitation and the "Ouzo effect": Application to drug delivery devices. *Advanced drug delivery reviews*. 2014;71:86-97.

[187] Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2006;2(1):8-21.

[188] Fessi H, Devissaguet JP, Puisieux F, Thies C. Procédé de préparation de systèmes colloïdaux dispersibles d'une substance, sous forme de nanoparticules. *French patent*. 1986;2:988.

[189] Ahmed N, Mora-Huertas C, Jaafar-Maalej C, Fessi H, Elaissari A. Polymeric drug delivery systems for encapsulating hydrophobic drugs. Drug Delivery Strategies for Poorly Water-Soluble Drugs. West Sussex,UK: *Wiley*; 2012. p. 151-97.

[190] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International journal of pharmaceutics*. 1989;55(1):R1-R4.

[191] Leroux J-C, Allémann E, Doelker E, Gurny R. New approach for the preparation of nanoparticles by an emulsification-diffusion method. *European journal of pharmaceutics and biopharmaceutics*. 1995;41(1):14-8.

[192] Quintanar-Guerrero D, Fessi H, Allémann E, Doelker E. Influence of stabilizing agents and preparative variables on the formation of poly (D, L-lactic acid) nanoparticles by an emulsification-diffusion technique. *International Journal of Pharmaceutics*. 1996;143(2):133-41.

[193] Quintanar-Guerrero D, Allémann E, Doelker E, Fessi H. Preparation and characterization of nanocapsules from preformed polymers by a new process based on emulsification-diffusion technique. *Pharmaceutical research*. 1998;15(7):1056-62.

[194] Moinard-Chécot D, Chevalier Y, Briançon S, Beney L, Fessi H. Mechanism of nanocapsules formation by the emulsion–diffusion process. *Journal of colloid and interface science*. 2008;317(2):458-68.

[195] Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with D, L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *Journal of Controlled Release*. 1993;25(1-2):89-98.

[196] Wehrle P, Magenheim B, Benita S. The influence of process parameters on the PLA nanoparticle size distribution, evaluated by means of factorial design. *European journal of pharmaceutics and biopharmaceutics*. 1995;41(1):19-26.

[197] Bodmeier R, Huagang C. Indomethacin polymeric nanosuspensions prepared by microfujidization. *Journal of Controlled Release*. 1990;12(3):223-33.

[198] Pisani E, Fattal E, Paris J, Ringard C, Rosilio V, Tsapis N. Surfactant dependent morphology of polymeric capsules of perfluorooctyl bromide: influence of polymer adsorption at the dichloromethane–water interface. *Journal of colloid and interface science*. 2008;326(1):66-71.

[199] Blanco M, Alonso M. Development and characterization of protein-loaded poly (lactide-co-glycolide) nanospheres. *European Journal of Pharmaceutics and Biopharmaceutics*. 1997;43(3):287-94.

[200] Lamprecht A, Ubrich N, Pérez MH, Lehr C-M, Hoffman M, Maincent P. Biodegradable monodispersed nanoparticles prepared by pressure homogenization-emulsification. *International Journal of Pharmaceutics*. 1999;184(1):97-105.

[201] Cohen-Sela E, Chorny M, Koroukhov N, Danenberg HD, Golomb G. A new double emulsion solvent diffusion technique for encapsulating hydrophilic molecules in PLGA nanoparticles. *Journal of controlled release*. 2009;133(2):90-5.

[202] Meng FT, Ma GH, Qiu W, Su ZG. W/O/W double emulsion technique using ethyl acetate as organic solvent: effects of its diffusion rate on the characteristics of microparticles. *Journal of controlled release*. 2003;91(3):407-16.

[203] Garti N. Double emulsions—scope, limitations and new achievements. Colloids and Surfaces A: *Physicochemical and Engineering Aspects*. 1997;123:233-46.

[204] Georgieva D, Schmitt V, Leal-Calderon F, Langevin D. On the possible role of surface elasticity in emulsion stability. Langmuir. 2009;25(10):5565-73.

[205] Krause H-J, Rohdewald P. Preparation of gelatin nanocapsules and their pharmaceutical characterization. *Pharmaceutical research*. 1985;2(5):239-43.

[206] Lertsutthiwong P, Noomun K, Jongaroonngamsang N, Rojsitthisak P, Nimmannit U. Preparation of alginate nanocapsules containing turmeric oil. *Carbohydrate Polymers*. 2008;74(2):209-14.

[207] Lertsutthiwong P, Rojsitthisak P, Nimmannit U. Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. *Materials Science and Engineering: C*. 2009;29(3):856-60.

[208] Lutter S, Koetz J, Tiersch B, Boschetti-de-Fierro A, Abetz V. Formation of gold nanoparticles in triblock terpolymer-modified inverse microemulsions. Colloids and Surfaces A: *Physicochemical and Engineering Aspects*. 2008;329(3):169-76.

[209] Bindschaedler C, Gurny R, Doelker E. Process for preparing a powder of water-insoluble polymer which can be redispersed in a liquid phase, the resulting powder and utilization thereof. *Google Patents*; 1990.

[210] Allémann E, Gurny R, Doelker E. Preparation of aqueous polymeric nanodispersions by a reversible salting-out process: influence of process parameters on particle size. *International Journal of Pharmaceutics*. 1992;87(1-3):247-53.

[211] De Jaeghere F, Allémann E, Leroux J-C, Stevels W, Feijen J, Doelker E, et al. Formulation and lyoprotection of poly (lactic acid-co-ethylene oxide) nanoparticles: influence on physical stability and in vitro cell uptake. *Pharmaceutical research*. 1999;16(6):859-66.

[212] Konan YN, Gurny R, Allémann E. Preparation and characterization of sterile and freeze-dried sub-200 nm nanoparticles. *International journal of pharmaceutics*. 2002;233(1):239-52.

[213] Nguyen CA, Allémann E, Schwach G, Doelker E, Gurny R. Synthesis of a novel fluorescent poly (D, L-lactide) end-capped with 1-pyrenebutanol used for the preparation of nanoparticles. *European journal of pharmaceutical sciences*. 2003;20(2):217-22.

[214] Zweers ML, Engbers GH, Grijpma DW, Feijen J. In vitro degradation of nanoparticles prepared from polymers based on DL-lactide, glycolide and poly (ethylene oxide). Journal of Controlled Release. 2004;100(3):347-56.

[215] Zweers ML, Engbers GH, Grijpma DW, Feijen J. Release of anti-restenosis drugs from poly (ethylene oxide)-poly (DL-lactic-co-glycolic acid) nanoparticles. *Journal of controlled release*. 2006;114(3):317-24.

[216] Chern C-S. Principles and applications of emulsion polymerization: *John Wiley & Sons*; 2008.

[217] Landfester K, Musyanovych A, Mailänder V. From polymeric particles to multifunctional nanocapsules for biomedical applications using the miniemulsion process. Journal of Polymer Science Part A: *Polymer Chemistry*. 2010;48(3):493-515.

[218] Norakankorn C, Pan Q, Rempel GL, Kiatkamjornwong S. Factorial experimental design on synthesis of functional core/shell polymeric nanoparticles via differential microemulsion polymerization. Journal of applied polymer science. 2010;116(3):1291-8.

[219] Barratt G. Characterization of colloidal drug carrier systems with zeta potential measurements. Pharm Technol Eur. 1999;11:25-32.

[220] Gamboa JM, Leong KW. In vitro and in vivo models for the study of oral delivery of nanoparticles. Advanced drug delivery reviews. 2013;65(6):800-10.

[221] Evans D, Pye G, Bramley R, Clark A, Dyson T, Hardcastle J. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut. 1988;29(8):1035-41.

[222] O'Neill MJ, Bourre L, Melgar S, O'Driscoll CM. Intestinal delivery of non-viral gene therapeutics: physiological barriers and preclinical models. Drug discovery today. 2011;16(5):203-18.

[223] Allen A, Snary D. The structure and function of gastric mucus. Gut. 1972;13(8):666.

[224] Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Advanced drug delivery reviews. 2012;64(6):557-70.

[225] Lai SK, Wang Y-Y, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Advanced drug delivery reviews. 2009;61(2):158-71.

[226] Mora-Huertas C, Fessi H, Elaissari A. Polymer-based nanocapsules for drug delivery. International journal of pharmaceutics. 2010;385(1):113-42.

[227] D'Souza S. A Review of In Vitro Drug Release Test Methods for Nano-Sized Dosage Forms. Advances in Pharmaceutics. 2014;2014.

[228] Heng D, Cutler DJ, Chan H-K, Yun J, Raper JA. What is a suitable dissolution method for drug nanoparticles? *Pharmaceutical research*. 2008;25(7):1696-701.

[229] Sanna V, Roggio AM, Siliani S, Piccinini M, Marceddu S, Mariani A, et al. Development of novel cationic chitosan-and anionic alginate-coated poly (D, L-lactide-co-glycolide) nanoparticles for controlled release and light protection of resveratrol. *Int J Nanomedicine*. 2012;7(2):5501-16.

[230] Verreck G, Chun I, Rosenblatt J, Peeters J, Van Dijck A, Mensch J, et al. Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer. *Journal of Controlled Release*. 2003;92(3):349-60.

[231] Zhang Y, Wang H, Li C, Sun B, Wang Y, Wang S, et al. A novel three-dimensional large-pore mesoporous carbon matrix as a potential nanovehicle for

the fast release of the poorly water-soluble drug, celecoxib. *Pharmaceutical research*. 2014;31(4):1059-70.

[232] Mahkam M, Hosseinzadeh F, Galehassadi M. Preparation of ionic liquid functionalized silica nanoparticles for oral drug delivery. 2012.

[233] Wallace SJ, Li J, Nation RL, Boyd BJ. Drug release from nanomedicines: selection of appropriate encapsulation and release methodology. *Drug delivery and translational research*. 2012;2(4):284-92.

[234] Yue P-F, Lu X-Y, Zhang Z-Z, Yuan H-L, Zhu W-F, Zheng Q, et al. The study on the entrapment efficiency and in vitro release of puerarin submicron emulsion. *Aaps Pharmscitech*. 2009;10(2):376-83.

[235] Panyam J, Sahoo SK, Prabha S, Bargar T, Labhasetwar V. Fluorescence and electron microscopy probes for cellular and tissue uptake of poly (D, L-lactide-co-glycolide) nanoparticles. *International journal of pharmaceutics*. 2003;262(1):1-11.

[236] Prasad S, Dangi J. Development and characterization of pH responsive polymeric nanoparticles of SN-38 for colon cancer. *Artificial cells, nanomedicine, and biotechnology*. 2015:1-11.

[237] Sun S, Liang N, Yamamoto H, Kawashima Y, Cui F, Yan P. pH-sensitive poly (lactide-co-glycolide) nanoparticle composite microcapsules for oral delivery of insulin. *International journal of nanomedicine*. 2015;10:3489.

[238] Henderson R, Benson J, Hahn F, Hobbs C, Jones R, Mauderly J, et al. New approaches for the evaluation of pulmonary toxicity: bronchoalveolar lavage fluid analysis. *Toxicological Sciences*. 1985;5(3):451-8.

[239] Price P, McMillan TJ. Use of the tetrazolium assay in measuring the response of human tumor cells to ionizing radiation. *Cancer research*. 1990;50(5):1392-6.

[240.   Dothel G, Vasina V, Barbara G, De Ponti F. Animal models of chemically induced intestinal inflammation: predictivity and ethical issues. *Pharmacology & therapeutics*. 2013;139(1):71-86.

[241] Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. 2013.

[242] Beloqui A, Coco R, Alhouayek M, Solinís MÁ, Rodríguez-Gascón A, Muccioli GG, et al. Budesonide-loaded nanostructured lipid carriers reduce inflammation in murine DSS-induced colitis. *International journal of pharmaceutics*. 2013;454(2):775-83.

[243] Dai C, Zheng C-Q, Meng F-j, Zhou Z, Sang L-x, Jiang M. VSL# 3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. *Molecular and Cellular Biochemistry*. 2013;374(1-2):1-11.

[244] Dharmani P, Leung P, Chadee K. Tumor necrosis factor-α and Muc2 mucin play major roles in disease onset and progression in dextran sodium sulphate-induced colitis. *PLoS One*. 2011;6(9):e25058.

[245] Laroui H, Viennois E, Xiao B, Canup BS, Geem D, Denning TL, et al. Fab'-bearing siRNA TNFα-loaded nanoparticles targeted to colonic macrophages offer an effective therapy for experimental colitis. *Journal of Controlled Release*. 2014;186:41-53.

[246] Fuss IJ, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *The Journal of clinical investigation*. 2004;113(10):1490-7.

[247] Martínez-Moya P, Romero-Calvo I, Requena P, Hernández-Chirlaque C, Aranda CJ, González R, et al. Dose-dependent antiinflammatory effect of ursodeoxycholic acid in experimental colitis. *International immunopharmacology*. 2013;15(2):372-80.

[248] Neurath MF, Fuss I, Kelsall BL, Stüber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *Journal of Experimental Medicine*. 1995;182(5):1281-90.

[249] Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA. Inflammatory mediators of experimental colitis in rats. *Gastroenterology*. 1989;97(2):326-37.

[250] MacPherson B, Pfeiffer C. Experimental production of diffuse colitis in rats. *Digestion*. 1978;17(2):135-50.

[251] Yalniz M, Demirel U, Orhan C, Bahcecioglu IH, Ozercan IH, Aygun C, et al. Nadroparin sodium activates Nrf2/HO-1 pathway in acetic acid-induced colitis in rats. *Inflammation*. 2012;35(3):1213-21.

[252] Schmidt C, Lautenschlaeger C, Collnot E-M, Schumann M, Bojarski C, Schulzke J-D, et al. Nano-and microscaled particles for drug targeting to inflamed intestinal mucosa—A first in vivo study in human patients. *Journal of controlled release*. 2013;165(2):139-45.