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# Drug and Cell Delivery Systems in the Treatment of Colitis

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## 1. Introduction

The term colitis describes variety of inflammatory diseases of the colon which can be differentiated according to their etiology, clinical, endoscopic and histological characteristics. In general, they can be classified as inflammatory bowel diseases (IBD) and infectious colitis (Hemstreet & Diprio, 2008; Novaneethan & Giannella, 2011), and non-IBD and non-infectious colitis, including ischemic colitis, chemical colitis, microscopic colitis, segmental colitis, radiation colitis, diversion colitis, eosinophilic colitis and Behcet's colitis (Koutroubakis, 2008). The two forms of idiopathic IBD, ulcerative colitis (UC), a mucosal inflammatory condition restricted to the rectum and colon, and Crohn's disease (CD), a transmural inflammation of the GIT affecting any part from the mouth to anus, are the most prevalent, especially in the western countries and in areas of northern latitude. Other forms, such as diversion colitis, eosinophilic colitis and Behcet's colitis are rare, with unknown ethiopathogenesis and limited epidemiological data. Clinical presentation of all forms is very similar and includes mild, moderate or severe local complications, such as diarrhea, abdominal pain or cramping, rectal bleeding and weight loss, and systemic ones, including hepatobiliary, joint, ocular, renal, dermatologic and mucosal complications.

The goals of the treatment include resolving of the acute inflammation and associated complications, alleviation of the systemic manifestations and maintenance of remission. Besides non-pharmacologic therapy, which includes nutritional support and surgical intervention, pharmacologic therapy is an integral part of the overall treatment of colitis. All the drugs are aimed to control the disease allowing the patient to perform normal daily activities. The main pharmacologic groups of drugs used for colitis treatment include aminosalicilates, corticosteroids, immunosuppressive agents, antimicrobials and inhibitors of TNF- $\alpha$ .

In addition, for maintaining remission in various GI diseases, including colitis, live bacterial cell biotherapeutics i.e probiotics, alone or combined with prebiotics as synbiotics, are also administered. Probiotics are defined as "viable microorganisms which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial effects in the host", while prebiotics as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" are considered (Gibson & Roberfroid, 1995). For prevention and maintaining of remission in colitis, various probiotics were clinically examined during the last decade, among which non-pathogenic *E. coli*, strains of bifidobacteria, lactobacilli, *Streptococcus*

*thermophilus*, enterococci, coliforms, *Bacteroides* and *Clostridium perfringens*. From the prebiotics, the commercially available fructooligosaccharides (FOS), inulin and galactooligosaccharides are frequently used and many other potential prebiotics are still under investigation, among which xylooligosaccharides, soy-oligosaccharides, pesticoooligosaccharides, glucoooligosaccharides, isomaltooligosaccharides and gentioooligosaccharides (Table 1).

Probiotic strain	Therapeutic effect	References
<i>B. bifidum</i> , <i>B. breve</i> , <i>B. infantis</i> , <i>B. lactis</i> LA 303, <i>B. longum infantis</i> UCC35624, <i>B. longum</i> , <i>L. acidophilus</i> LA 201, <i>L. acidophilus</i> , <i>L. casei</i> Shirota, <i>L. casei</i> subsp. <i>Rhamnosus</i> , <i>L. delbruecki</i> subsp. <i>Bulgaricus</i> , <i>L. fermentum</i> BR11, <i>L. plantarum</i> 299V, <i>L. plantarum</i> LA 301, <i>L. paracasei</i> , <i>L. reuteri</i> , <i>L. rhamnosus</i> GG, <i>L. salivarius</i> LA 302, <i>L. salivarius</i> UCC118, <i>Lactococcus lactis</i> , non-pathogenic <i>E. coli</i> Nisle 1917, <i>Sacharomyces boulardi</i> , <i>Str. boulardi</i> , <i>Str. salivarius</i> subsp. <i>Thermophilus</i>	Maintain balance of beneficial <i>vs.</i> aggressive commensal enteric microflora: inhibit pathogenic enteric bacteria (decrease luminal pH, secrete bactericidal proteins, resist colonization, block epithelial binding-induction of MUC2, inhibit epithelial invasion-Rho (in)dependent pathways); improve function of epithelial and mucosal barrier (produce short chain fatty acids (SCFAs), enhance mucus production, increase barrier integrity); alter immune-regulation (induce IL-10 and transforming growth factor $\beta$ expression and secretion, stimulate secretory IgA production, decrease tumor necrosis factor expression).	Geier et al., 2007; Peran et al., 2007; Prakash, 2008.
Prebiotic		
Inulin, lactulose, goat's milk oligosaccharides, fructooligosaccharide, hemi-celluloses- and glutamine-rich extract, maltodextrin	Decrease level of the pro-inflammatory cytokine IL-1 $\beta$ , increase anti-inflammatory TGF- $\beta$ , increase caecal lactobacillus and bifidobacterium levels, decrease <i>E. coli</i> colonization, decrease clostridium and enterobacterium levels, increase levels of SCFAs.	Geier et al., 2007; Gibson & Roberfroid, 1995.

Table 1. Health benefits of different probiotic strains and prebiotics in colitis

Considering the pharmacologic treatment, many patients experience significant undesired effects (Table 2) which require discontinuation of the therapy. Avoiding or minimizing these effects is one of the great challenges in the pharmaceutical industry in which great effort is put on design of an ideal drug delivery system that would deliver the drug at a rate dictated by the needs of the patient within the period of treatment and target it to the specific i.e. inflamed site of the colon. Considering probiotics, the greatest achievement with these advanced delivery systems is their potential to protect the probiotics not only in the pro- or syn-biotic food or pharmaceutical product, but also from the harsh environment of the GIT, and to maintain their functionality unaltered on arrival to the colon. All these prerequisites require modified drug and/or cell release technologies which can improve the therapeutic efficacy and safety by precise temporal and spatial placement in the colon, thereby reducing both the dose and the frequency of administration.

Drug	Adverse effects	References
<b>Aminosalicylates</b>		
Sulfasalazine Mesalamine (5-Aminosalicylic acid; 5-ASA)	Agranulocytosis, pancreatitis, interstitial nephritis, hepatitis, male infertility, arthralgia, pneumonitis. Mesalazine derivatives manifest lower frequency of adverse effects in comparison with sulfasalazine.	Hemstreet & Diprio, 2008; Linares et al., 2011; Sonu et al., 2010.
<b>Corticosteroids</b>		
Budesonide Prednisone Prednisolone Dexamethasone	Hyperglycemia, hypertension, electrolyte disturbances, cataracts, osteoporosis, myopathy, conditions associated with immune suppression, adrenal insufficiency (long-term administration).	Ford et al., 2011.
<b>Immunosuppressive agents</b>		
Azathioprine Methotrexate Cyclosporine	Pancreatitis, fever, rash, arthralgia, diarrhea, infectious complications, hepatitis, myelosuppression, known carcinogen. Diarrhea, skin reactions, bone marrow suppression, lung lesions, kidney dysfunction, hepatotoxicity, folic acid deficiency. Paresthesias, hypertension, nephrotoxicity, seizures.	Gisbert et al., 2009; Hemstreet & Diprio, 2008; Wahed et al., 2009.
<b>Antimicrobial agents</b>		
Metronidazole Vancomycin Ciprofloxacin Tobramycin	Urticaria, glossitis; long-term use may develop paresthesia, reversible peripheral neuropathy. Infusion related events, nephrotoxicity, pseudo-membranous colitis, ototoxicity, reversible neutropenia, infrequently anaphylaxis, eosinophilia, rashes including exfoliative dermatitis, linear IgA bullous dermatosis, Steven-Johnson syndrome, vasculitis. Diarrhea, vomiting and rash. Other side effects (e.g. headache, abdominal pain, pain in extremities, injection site reaction, cardiovascular, gastrointestinal, etc.) in less than 1% of the patients. Ototoxicity, nephrotoxicity, neurotoxicity, anemia, granulocytopenia, thrombocytopenia, fever, rash, exfoliative dermatitis, itching, urticaria, diarrhea, headache, lethargy, pain at the injection site, mental confusion, disorientation.	Hemstreet & Diprio, 2008; Khan et al., 2011.
<b>Inhibitors of TNF-<math>\alpha</math></b>		
Infliximab Adalimumab Etanercept Certolizumab	Acute infusion reactions, serum sickness, increase in serious infections (e.g. sepsis, pneumonia, tuberculosis), worsening of existing heart failure and even death.	Tursi et al., 2010; Talley et al. 2011.

Table 2. Adverse effects of drugs most commonly used for the treatment of colitis

## 2. Colon as a target for drug and probiotic cell delivery in colitis; biological and (patho)physiological factors

The principal goals of colon-specific delivery after oral administration are to prevent biodegradation of drugs and maintain viability of the probiotic cells in the stomach and small intestine where acid- or enzyme-labile drugs and cells are degraded, to avoid absorption of drugs in the upper intestine and accordingly, to release the drugs or to provide colonization of the cells in the lower intestine. Motility of the GIT, high surface area of the small intestine, pH of the intestinal fluids, bacterial flora, they all can affect and to a certain instance be an obstacle for efficacious colon targeted and controlled drug/cell delivery after oral administration. The segmental contractions of the colon increase the contact with the mucosa, which in turns promotes the design of mucoadhesive drug delivery systems for colon targeted and prolonged drug/cell release. Colonic transit time of a single-unit delivery systems varies significantly within a day; app. 6 hours are needed for the form to reach the transverse colon in the morning at fasting state, while in the evening, the colonic transfer is slower and app. 11 hours are needed for the dosage form to reach the transverse colon. The transit time from the stomach to the large intestine is 2-4 h and from the small intestine to the anus 6-48 h. This transit time may be altered by many factors, such as age, sex, dietary and disease factors (Washington et al., 2001).

The pH in the GIT ranges from 1.3-1.7 in the resting human stomach to 6.4 in duodenum, and then drops to the range of 5.0-6.5. In the colon, pH ranges from  $6.4 \pm 0.6$  in the ascending part to  $6.6 \pm 0.8$  in the transverse colon and  $7.0 \pm 0.7$  in the descending part (Washington et al., 2001). The literature data related to the colonic pH values in the state of colitis are controversial, pointing to increase, decrease or no change of pH at all (Nugent et al., 2001). Unpredictable alteration of the pH profile may significantly affect the viability of the cells and local bioavailability of the drugs by changing their chemical stability and degree of ionization i.e. absorption. This effect is particularly emphasized when delivery systems composed of pH sensitive polymers as drug or cell carriers for colon targeted and controlled release are used.

The total metabolic activity of the colonic wall is much lower than the one in the upper gut, so, the enzymatic degradation of drugs and cells is insignificant. However, the low redox potential favors the growth of low number of fungi and around  $10^{12}$  viable bacteria/g of large bowel content in human. App. 400-500 bacterial are present, dominantly obligate anaerobic species which produce enzymes for lot of metabolic reactions that affect the drug and cell release from their delivery systems. This effect is especially emphasized when biodegradable polymers/systems are used. The predominant anaerobic species in the colon are *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, whilst facultative aerobes are represented by *E. coli*, *Klebsiella*, *Streptococcus*, *Staphylococcus*, *Bacillus* and *Lactobacillus*. The enzymes they produce are  $\beta$ -glucuronidase,  $\beta$ -galactosidase, nitroreductase, azoreductase, etc. Number of mucosal bacteria increases progressively in the state of inflammation, with concentrations relatively higher in patients with active disease (Prakash & Urbanska, 2008).

The goblet cells, which together with absorptive and endocrine cells make up the colonic epithelial layer, are responsible for the production of mucus. In the inflamed state, patients manifest reduced thickness of the colonic mucus layer due to the reduced number of the



goblet cells. The mucus layer is nearly free of bacteria in the mid to distal murine colon, but this is not true for the mucosa-adjacent and luminal regions of the caecum and proximal colon. Microorganisms co-aggregate and form biofilms that adhere to the epithelial surface (Strugala et al., 2008). Fast mucus turnover in colitic patients followed by increased activity of the bacterial enzymes and high concentrations of positively charged amino acids in the peptide core of the mucins may affect the affinity of charged drugs and cells and/or drug and cell delivery systems towards mucosa. Electrostatic interactions that occur may intensify adhesion to the inflamed mucosa and prolong the residence time of the delivery system or drug or cell in the colon. This effect is especially emphasized when bioadhesive polymers as drugs and cell carriers are used. Providing intimate contact with the mucosa, systems composed of bioadhesive polymers become resistant to GI motility, whilst the drug or cell release rate is controlled by the polymers' hydration, erosion and biodegradation.

It is well known that adaptive immune system relies on the lymphocytes, which are organized in Peyer's patches and isolated follicles. M-cells, a constitutional part of the follicle-associated epithelium, are responsible for sampling of particulate materials, including microbial cells. In inflamed mucosa, M cells get damaged and increased, which can significantly affect the selective retention of the small particles in the colon. This is especially significant when multi-particulate dosage forms, with different size distribution, as drug or cell carriers are administered (Washington et al., 2001).

Considering above mentioned, a design and development of advanced drug and cell carrier systems that react exclusively to the conditions in the colon and deliver their content with a controlled rate is of paramount importance. These systems can be optimized by using pH-sensitive and biodegradable polymers that provide selective adhesivity to the colonic mucosa. Delivery to the proximal colon may be achieved only when these systems remain intact for app. first 5 hours after administration and release their content within 10-24 hours.

### **3. Drug and cell delivery systems in colitis; strategies for targeted and controlled delivery**

Advanced systems for colon-targeted and controlled drug and cell delivery in colitis are designed to modify drug and cell release and induce desired local effect by releasing the drug/cells in high concentrations close to the disease area, thereby minimizing systemic side effects. With these modified release dosage forms, not only therapeutic, but also safety and convenience objectives are accomplished, which are not typical for the conventional dosage forms. In conventional (non-parenteral) delivery, when using so called immediate-release dosage forms, blood concentrations of the drug rise after drug administration, than peak and decline. In such dosage forms, only the dose and dosing interval can vary and, for each drug, there exists a therapeutic window of plasma concentration below which the therapeutic effect is insufficient and above which toxic side effects occur.

An ideal form of colon drug delivery is a sustained or controlled form of drug release, which provides extended release, keeps plasma concentrations constant within the therapeutic window, and in this way, reduces dosage frequency at least two fold in comparison with the immediate release dosage form. The goal of the controlled release dosage forms is usually accomplished by attempting to obtain zero-order drug release. Drug carriers generally do not achieve this type of release considering lot of difficulties in dosage form design and

production, but providing drug release in a slow first-order fashion results also in prolonged therapeutic effect. Delayed release dosage forms (e.g., enteric coated forms) are designed to release the drug at a time different than immediately after administration. The delay may be controlled by the influence of the environmental factors (e.g., GI pH, bacteria, temperature, pressure, etc) and time-controlled, such as in pulsatile release systems. The pressure controlled colon delivery utilizes the increase in pressure of the luminal contents in the colon due to the reabsorption of water. pH-sensitive delivery utilizes solubility of the drug carrier in the luminal content of the colon, while in bacteria dependant delivery, colonic bacteria are utilized to degrade the drug/cell carrier. In pulsatile release systems, a complete and rapid release follows lag time. They are generally designed according to the circadian rhythm of the body with an aim to deliver the active ingredient at the right site of action, at the right time and in the right amount. In the colon delivery, this approach is based on principle of delaying the time of release of about 5 hours.

### 3.1 Conventional topical dosage forms

Rectal installation is an established approach for the treatment of the disease distally located up to the sigmoid descending junction. It has the advantage of shortest distance to the colon; however, it is inconvenient and followed by difficulties in reaching the proximal colon (Table 3). In general, suppositories, foams and liquid enemas as dosage forms are used. The selection of the type of the rectal preparation depends on the proximal extent of inflammation, ease of insertion and patient preference. Suppositories or foams reach about 15 to 20 cm, while liquid enemas distribute to about 30 to 60 cm (to the splenic flexure) and sometimes as far as the ascending colon (Washington et al., 2001). So, suppositories are generally indicated for the disease located to the rectosigmoid junction, whereas foam enemas are usually distributed to the proximal sigmoid colon. In most patients, liquid enemas can deliver the drug as proximal as the splenic flexure. Foam and liquid enemas appear to be equally effective in treating patients with proximal UC, however, foam enemas are preferred because their administration is more easier and retention is more comfortable. Suppositories are usually better tolerated than enemas (Travis et al., 2008). Instilled volume and the viscosity of the enema are the most important variables defining proximal spreading of drugs. As large is the volume and the viscosity, more consistent is the proximal coating.

Rectal preparations of 5-ASA and corticosteroids are used as preferred treatment for mildly to moderately active left-sided or distal UC. The mechanism of action of 5-ASA is very complex and includes inhibition of cyclooxygenase and lipoxygenase, blocked production of leukotrienes and prostaglandins, inhibition of adenosine-induced secretion and bacterial peptide-induced neutrophil chemotaxis, scavenging of reactive oxygen metabolites and inhibition of activation of nuclear regulatory factor kappa B (Hemstreet & Diprio, 2008). Meta-analyses of clinical trials point to superior effect of rectal 5-ASA in comparison with placebo and conventional rectal corticosteroids in inducing remission of distal UC, which indicates the use of rectal steroids as reserves for 5-ASA when treatment with amino-salicylates failures or intolerance occurs (Marshall et al., 2010). When efficacy and convenience of administration of different rectal 5-ASA formulations were compared, no significant difference in efficacy was observed, while foams and gels were evaluated as the most convenient, producing less abdominal bloating. Considering adverse effects, reduced rate in respect to oral administration was reported (Sonu, 2010).

In colitis, corticosteroids are believed to modulate the immune response and inhibit production of cytokines and mediators being the benchmark therapy for moderate to severe UC and CD. Because of their adverse effects (Table 1), they should be used only in short term to induce remission in active UC, stopped once remission has been achieved and gradually discontinued. As selection criteria for rectal administration, corticosteroids with high efficacy and low systemic concentration are preferred, in order first-pass effect and adrenal suppression to be minimized and other adverse effects as well (Hanauer, 2002). Rectal formulations of prednisolone-metasulfofenzoate, budesonide, fluticasone, tixocortol pivalate and beclomethasone dipropionate are commercialized due to their lower interference with the adrenocortical function in comparison with hydrocortisone acetate and betamethasone (Gionchetti et al., 2004; Hanauer, 2002). Budesonide has been the corticosteroid of choice marketed as foams, liquid enemas and suppositories. It manifested efficacy in the induction and short-term maintenance of CD and induction of remission in collagenous and microscopic colitis (O'Donnell, 2010). Comparison of the efficacy, tolerability, safety and patient's preference of budesonide foam *vs.* enema pointed to no significant difference in efficacy and safety and confirmed better tolerability and easier application of the foam formulations (Gross et al., 2006).

The use of immunosuppressant drugs is effective for long-term treatment of UC and CD. These agents are generally reserved for patients refractive to steroids and they are associated with serious adverse effects, which are potentiated with their relatively long-term use (Table 1). Rectal foams of azathioprine have been patented (Sandborn, 2002) and in one study in which healthy human subjects were included, pharmacokinetics of azathioprine after intravenous, oral, oral delayed release and rectal foam was compared. Rectal foams considerably reduced systemic 6-mercaptopurine bioavailability indicating the possibility for limited toxicity by local delivery of high doses of azathioprine (Van Os et al., 1996).

### 3.2 Advanced drug and cell delivery systems

Oral route is more convenient in the treatment of colitis. However, it is the longest one and associated by lot of obstacles for drug and cell stability and achieving high concentrations in the colon. Various strategies have been used to overcome these obstacles and to avoid high systemic bioavailability. These approaches utilize either formulation-specific or (pro)drug-specific design, while drug/cell targeting and modified release can be achieved by one or more of the well-established mechanisms: pH-sensitive, time-dependent, pressure-dependent and bacteria-dependent delivery (Table 3).

Principle	Advantages	Disadvantages
<b>Conventional, topical delivery systems</b>		
Deliver drugs by rectal instillation of liquid enemas, foams and suppositories	Deliver therapeutic drug concentrations to the distal regions of the colon Limited systemic toxicity Drug is protected from digestion	Difficulties in reaching the proximal colon Inconvenient administration and local irritation (e.g., leakage, problems with retention, burning sensation and bloating)



Principle	Advantages	Disadvantages
		Less compliance than optimal Not suitable when high doses are required
<b>Advanced delivery</b>		
<i>pH-responsive delivery systems</i>		
Release active ingredient in response to the change in pH throughout the GIT utilizing enteric polymers with high pH threshold	Provide uniform and prolonged release of the active ingredient throughout the intestinal region specifically at the diseased site Maintain physical and chemical integrity of the drugs in the GIT Preserve cell viability above therapeutic value during the passage through the stomach	Possibility for premature release of the active ingredient in the upper GIT and loss of therapeutic efficacy (e.g., rupture of the coating in the stomach, etc.) Failure of the enteric coating to dissolve at the desired site of action (e.g., formulation error, reduced colonic pH due to the presence of SCFAs, residue of bile acids, CO <sub>2</sub> , etc.) Uncertainty of the location of the active ingredient release due to the variability in gut pH in colitis (e.g., reduced pH in UC, unknown pH in CD, etc.)
<i>Bacteria-triggered delivery systems</i>		
Release active ingredient in response to the specific enzymatic activity of the microflora present in the colon by biodegradation of the drug/cell carrier	Precise and direct effect at the diseased site of the colon (colon-targeted delivery) Maintain stability of drugs/viability of cells in the upper GIT Control drug/cell release Lower the required dose and frequency of administration Have minimal effect/lower toxicity on the rest of the body Flexibility in design (e.g., prodrug design, CODES™, TARGIT®, COLAL™, etc.)	Risk of producing harmful substance as a product of carrier degradation (e.g. azo-polymer-based formulations) Difficulties in attaining desired rate of drug/cell release (e.g., rapid swelling in the upper GIT, fast disintegration or excessive slow enzymatic degradation of the delivery system) Inconsistency in drug/cell release due to the factors that might affect degradation of the delivery system (e.g. dietary fermentation pre-cursors, type of food consumed, co-administration of chemotherapeutic agents, etc.) In a case of prodrug, new

Principle	Advantages	Disadvantages
		chemical entity needs additional evaluation before being used as a carrier
<i>Time-dependent delivery systems</i>		
Release active ingredient after a predetermined lag time (5-6 hours) utilizing enteric coating to withstand the variations in gastric emptying time and pH	Deliver drug at preselected time or pre-selected site of the GIT Delay or sustain drug release Maintain physical and chemical integrity of the drug in the stomach Lower the required dose and frequency of administration Reduce side-effects Integrate pH-sensitive and time-release functions into a single dosage form	Time-specific delivery cannot be accurately predicted due to the inter- and intra-individual variations in gastric emptying time, peristalsis or contraction in the stomach and type and amount of food consumed Delivery can be affected by the symptoms of colitis (e.g., accelerated transit time through different regions of colon due to diarrhea, etc.)
<i>Osmotic-controlled delivery systems</i>		
Release active ingredient utilizing osmotic pressure with a 3-4 hour post gastric delay	Release drug with pre-determined zero order rate Target drug locally to the colon Deliver drug independently of the physiological factors in the GIT Suitable for delivery of drugs with moderate water solubility Versatile designs deliver drug as short as 4 hours or provide constant release for up to 24 h Reduce side-effects Lower the required dose and frequency of administration	Delivery can be, to a certain degree, affected by administered food Delivery varies with the gastric motility Irritation or ulcer may occur due to release of saturated solution of drug More expensive treatment (because of multiple formulation steps and necessity of using special equipment for making an orifice in the system)
<i>Pressure-controlled delivery systems</i>		
Release active ingredient in response to the increased luminal pressure caused by the strong peristaltic waves in the colon	Colon-specific delivery Drug release mechanism is independent of pH Reduce side-effects Lower the required dose and frequency of administration	Limited understanding of the raised pressure phase in subjects with colitis Unpredictable delivery in the fed state because of the contractions in the stomach that may disintegrate the system in the stomach

Table 3. Characteristics of various approaches for drug and cell delivery in colitis

### 3.2.1 pH-dependent systems

Colonic formulations are very similar to conventional enteric-coated formulations, but consisted of enteric polymers with ability to withstand an environment ranging from low to neutral pH and stay intact for minimum 5 hours. In order to prevent premature drug release in the upper intestine, a combination of polymers with different solubility properties and water permeability/hydration rate and/or higher coating levels of enteric polymers are applied, thereby taking care coats not to rupture. The amount of coating depends on the solubility characteristics of both, the drug and the polymer(s), desired release profile and type of the final dosage form. Most commonly used coating polymers are derivatives of acrylic acid, methacrylic acid copolymers, known as Eudragit®S, Eudragit®L and Eudragit®S, copolymers of methacrylic acid and methyl methacrylate, Eudragit®L-100 and Eudragit®S-100, Eudragit FS, Eudragit P4135 F, and derivatives of cellulose in a form of salts, such as hydroxypropylmethyl-cellulose phthalate (HPMCP 50 and 55), cellulose acetate phthalate (CAP), etc., generally, with threshold pH above 4.8 to 7.0. In respect to formulation, coated dosage forms may be either single-unit or multi-particulate systems formulated as a single- or multi-layer product. The coating can be applied to a wide variety of solid core formulations such as (mini)tablets, capsules, pellets, granules, micro- and nano-particles. Most of them can be further filled into gelatin capsules or compressed as tablets, which can be additionally coated with the same or different suitable enteric polymer. The multi-particulate forms are less affected by the variations in the GIT, have larger surface, greater potential for homogenous spreading and reproducible drug release in the inflamed sites of the colon (Chourasia & Jain, 2003; Singh, 2007).

5-ASA tablets coated with Eudragit®L-100 are commercially available as Claversal™, Salofalak™, Mesasal®, Calitofalk® and Rowasa®, while sulfasalazine tablets coated with Eudragit® L-100-55 and CAP as Colo-pleon® and Azulfidine®, accordingly. They can effectively deliver 5-ASA to the terminal ileum and proximal colon in patients with IBD, with a delayed release which is achieved by a relatively thick coating. Clinical studies indentified a mean disintegration time of 3.2 h after gastric emptying and possibility the drug release to start at pH 6.6. In order 5-ASA release to be delayed and release to start at pH above 7.0, Eudragit®S-100 was used and the prepared delayed-release tablets were marketed as Asacol® (Schroeder et al., 1987). In January 2007, first 5-ASA formulation for once-a-day dosing was approved (Lialda™, Mezavant™). It uses a patented multi-matrix system, whereby the 5-ASA is incorporated into microparticles of a lipophilic matrix dispersed within a hydrophilic matrix. This is coated by a gastroresistant polymer which breaks down at pH of 7.0, allowing controlled release and delayed degradation of the 5-ASA in the colon. Multi-particulate forms of 5-ASA pellets coated with a combination of different Eudragits® (Eudragit® FS 30D, Eudragit® L-100, Eudragit® S-100) and Eudragits® with derivatives of cellulose (e.g. EC, microcrystalline cellulose, HPMC) were also prepared to achieve site specific release close to the ileocaecal valve. Rapid release at pH above 7.5 was observed, between 6.8 and 7.2 drug release was found to be zero order, while below 6.5 no release occurred (Cheng et al., 2004; Di Pretoro et al., 2010). Makham & Vakhshouri (2010) prepared and characterized methacrylic acid/perlite composites loaded with 5-ASA. In pH 7.4, with completed ionization, hydrolysis rate of the polymer was increased resulting in significant drug release.

For budesonide, similar pH-based systems, generally multi-particulate in a form of coated pellets or granules filled in gelatin capsule, are also commercially available (Budenofalk® and Entocort®EC) and patented (Beckert et al., 2005). In Budenofalk®, colon targeting and delayed release is accomplished by using ammonio methacrylate copolymer (Eudragit®RL), ammonio methacrylate copolymer (Eudragit®RS) and Eudragit®L-100 and Eudragit®S-100 for coating of granules with budesonide. In Entocort®EC, coating of the granules dissolves at pH>5.5 when they reach the duodenum. Thereafter, a matrix of EC with budesonide controls the release of the drug in a time-dependent manner. Budesonide was also efficiently entrapped in a micro-particulate system consisted of drug loaded acetate butyrate microspheres coated by Eudragit®S. No drug was released below pH 7 (Rodriguez et al., 1998). Similar results were obtained when budesonide-layered pellets were coated with an inner layer of a combination of Eudragit®RL PO and RS PO and an outer layer of Eudragit FS (Patel et al., 2010). Also, novel pH-sensitive budesonide loaded nanospheres designed for colon-specific delivery were prepared using polymeric mixtures of poly (lactic-co-glycolic) acid (PLGA) and methacrylate copolymer. They showed strongly pH-dependent drug release properties in acidic and neutral pH followed by a sustained release phase at pH 7.4. In addition, superior therapeutic effect in alleviating the conditions of induced colitis in animal model was observed (Makhlof et al., 2009).

Colon targeted drug delivery systems based on methacrylic resins and/or cellulose derivatives has also been described for prednisolone (Thomos et al., 1985), beclomethazone dipropionate (Levine et al., 1987), dexamethasone (Wang et al., 2010), cyclosporine (Kim et al., 2001), quinolones (Van Saene, 1986), metronidazole (Obite et al., 2010) and azathioprine (Kotagale et al., 2010). In the study of Kotagale et al. (2010), coated tablets with azathioprine exploiting different polymer combinations of Eudragit-S®, Eudragit-L® and CAP were prepared. Desired release pattern was achieved with only 9.75% drug release in the first 5 h. Tacrolimus has been also formulated in colon delivery system. Namely, PLGA nanoparticles containing the drug were entrapped into pH sensitive microspheres, showing strongly pH-sensitive release kinetics of both nanoparticles and the drug (Lamprecht et al., 2005).

### 3.2.2 Bacteria-dependent systems

Microbially controlled delivery is the most utilized and probably the most site-specific approach for colon targeting of drugs and cells because it relies on drug/cell carriers that are recalcitrant to the conditions of the stomach and upper intestine. When reaching the colon, these materials undergo degradation by enzyme or break down of the polymer backbone, which leads to reduction in their molecular weight, loss of mechanical strength and subsequent drug/cell release with a rate that correlates with the biodegradation rate. For this type of drug/cell delivery, synthetic and natural polymers are used utilizing prodrug or multi-particulate approach. Multi-particulate approach has been utilized for oral delivery of sulfasalazine and betamethasone, based on microparticles of different synthetic biodegradable (co)polymers i.e. poly(epsilon-caprolactone), polylactic acid and PLGA (Lamprecht et al., 2000). However, the most of the multi-particulate systems, especially those carrying probiotic cells, utilize natural, generally regarded as safe (GRAS) polysaccharide polymers. Their fermentation by the bacterial enzymes results in formation of volatile SCFAs, such as lactic, acetic, propionic and butyric acids. Knowing that their deficiency causes UC, one can postulate that administering probiotics alone or with

prebiotics, with complex carbohydrate structure, or embedded in polysaccharide carriers could be significantly beneficial in the treatment of colitis.

### i. Polysaccharide-based systems

Lot of advantages promote the use of polysaccharides as drug carriers for colon-targeted and controlled delivery, such as wide availability and inexpensiveness, variety of structures, simplicity for (bio)chemical modification, stability, safety, non-toxicity, mucoadhesivity, pH sensitive solubility and gel-forming properties. Of polysaccharides, guar gum, inulin, chitosan, chondroitin sulphate, alginates and dextran are the most used (Kumar et al., 2009). However, these materials are with certain limitations. Their hydrophilic nature makes them either soluble or prone to swelling in an aqueous environment and hence unsuitable as drug or cell carriers. So, when they are used alone, large quantities are needed to target the colon and control the drug release. To overcome these problems, cross-linking of soluble polysaccharides with poly- or di-valent cations or anions, accordingly, to form insoluble salts, or coating with mucoadhesive and oppositely charged pH sensitive polymers is applied. In this way, combined mechanisms for colon targeting, controlling drug release and increasing mean residence time in the colon are utilized.

For 5-ASA delivery, colon specificity has been achieved using a system based on amylose (COLAL™), which is susceptible to digestion by amylase-producing bacteria present in the colon. To control the swelling in the aqueous media and in that way, 5-ASA release rate, pellets were coated with amylose coating solution prepared along with the hydrophobic polymers Ethocel®, Eudragit RS/RL 30D and Aquacoat ECD30 (Milojevic et al., 1996). In addition, successful colon delivery of prednisolone metasulfobenzoate with COLAL™ system in patients with active UC was reported (Thompson et al., 2002). Similarly, dispersion of pectin in EC was used as the film former for coating of 5-ASA pellet cores. Negligible drug release during first 5 h in the simulated gastric and small intestinal conditions was observed. Osmotically driven release and formation of channels in the film caused by dissolution of pectin and activated by the presence of rat caecal contents was proposed as a drug release mechanism (Wei et al., 2008). Similar results have been obtained when a tablet systems based on swelling matrix core containing pectin, HPMC, microcrystalline cellulose and 5-ASA was developed in which drug release rate was controlled by pectinases (Talukder & Fasihi, 2008). The systems were designed based on GI time concept, assuming colon arrival time of 6 h.

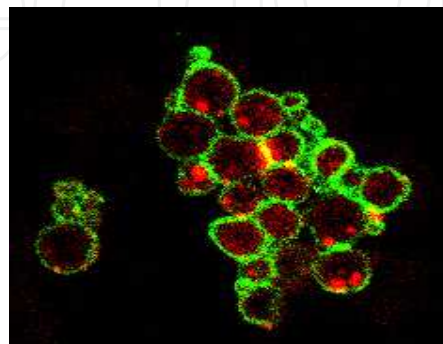


Fig. 1. Confocal laser scanning microscopy of 5-ASA loaded chitosan-Ca-alginate microparticles showing FITC-labeled chitosan (green) coating RBITC-labeled Ca-alginate matrix (red) (Mladenovska et al., 2007a,b).



Chitosan has also been extensively exploited as a 5-ASA carrier. For example, specific release of 5-ASA in the colon was achieved with chitosan capsules coated with HPMCP as enteric solvent material; efficacy in induced colitis in rats was confirmed as well as superiority in respect to the commercial 5-ASA products (Tozaki et al., 2002). 5-ASA loaded chitosan microspheres showing colon specific and controlled release were also prepared by Zambito & Di Colo (2003). In our studies (Mladenovska et al., 2007a,b), chitosan-Ca-alginate microparticles (Fig. 1) for colon-specific delivery and controlled release of 5-ASA after oral administration were prepared. *In vitro* drug release studies carried out in simulated *in vivo* conditions and biodistribution studies performed in colitic rats confirmed the potential of the particles to release the drug in the colon, with low systemic bioavailability. Similarly, beads containing 5-ASA, Eudragit FS 30D, Eudragit S-100 and chitosan were prepared (Iruin et al., 2005).

Corticosteroids were also incorporated in polysaccharide-based colon delivery systems. Multi-particulate system showing specific biodegradability and pH-dependent triamcinolone release were prepared based on chitosan, amidated pectin, HPMCP and CAP. Only 1% of drug was released in the acidic media after 2h (Oliveira et al., 2010). Novel colon delivery system COLAL-PRED has been developed by Alizyme for the treatment of UC as a combination of Alizyme's proprietary colonic drug delivery system COLAL and prednisolone sodium metasulfobenzoate. The product has a coating that breaks down only in the colon by locally present bacteria, thus increasing local drug delivery without significant systemic side effects (Rangasamu, 2010). Budesonide was microencapsulated with dextran and the formulation was *in vitro/in vivo* characterized in induced colitis in rats. Colon targeting was confirmed and the macroscopic damage and total colitis scores were significantly reduced in comparison with the control group receiving 5-ASA and budesonide suspension (Varshosaz et al., 2011a). When budesonide release from directly compressed matrix tablets prepared of different molecular weights of dextran was evaluated, app. 10% of the drug was released in acidic pH and pH 7.4, while a very drastic increase was observed after exposure to pH 6.8 containing rat caecal contents (Ahmadi et al., 2011). Budesonide loaded chitosan-Ca-alginate microparticles coated with Eudragit S-100 were also prepared showing sustained release in pH 2.0 and 6.8 and efficient release in pH 7.4 controlled by the erosion and biodegradation rate of the polymer matrix. Clinical and histological evaluation in rat model of colitis showed that colitis severity was significantly suppressed (Crcarevska et al., 2009).

A multi-particulate system combining pH-sensitive property and specific biodegradability for colon-targeted delivery of metronidazole has been also investigated. The system was prepared by coating cross-linked chitosan microspheres with Eudragit L-100 and S-100. No release was observed at acidic pH, but in the presence of rat caecal contents, significant release was observed, indicating the susceptibility of chitosan matrix to colonic enzymes (Chourasia & Jain, 2004). Pectin microspheres were also prepared and coated with Eudragit® S-100 showing continuous release of metronidazole at colonic pH in the presence of rat caecal contents (Vaidya et al., 2009). In the studies of Nasra et al. (2007), pectin as a carrier of metronidazole was combined with chitosan in a form of coated tablets with ability to prevent premature drug release.

Azathioprine loaded Ca-gellan beads coated with Eudragit®S-100 were also prepared. The results suggest that gellan gum undergoes significant degradation in the presence of

galactomannanase, which in turn facilitates the drug release from beads in the simulated colonic fluid (pH 7.4) in a controlled manner (Singh et al., 2004). In the work of Chaurasie et al. (2008), Ca-pectinate microspheres were prepared to deliver methotrexate in the colon. *In vitro* drug release studies in simulated gastric and intestinal fluids showed that app. 8% of the drug was released in 5 hours, whereas most of the loaded drug was released in simulated colonic fluid containing pectinase.

Polysaccharides have been also investigated as carriers for protection of the probiotics. Entrapment of cells in a gel matrix of alginates, chitosan, gellan, *k*-carageenan and starch or mixture of polysaccharide and protein is the most utilized approach. Cells are either compressed into a pellet, which is then encapsulated with the coating material by further compression, or encapsulated in an inner core surrounded by a semi-permeable, spherical, thin and strong membrane to form microcapsules or immobilized within or throughout a polymer matrix to form microspheres which can be subsequently filled into gelatin capsule. The coating of the microparticles, with a diameter from few microns to 1 mm, is designed to withstand acidic conditions and open in the lower intestine to release the cells by many different mechanisms, including fracture by heat, solvation, diffusion, pressure, erosion and biodegradation. The lower intestine provides right conditions for probiotic to survive, multiply and exert health beneficiary effects. With such a protection from acidity, molecular oxygen, hydrogen peroxide, digestive enzymes, bacteriophages and SCFAs, the viability of the probiotic after oral administration is significantly improved and targeted and controlled release achieved (Rokka & Rantamaki, 2010).

Literature data point to abundance researches related to microencapsulation of probiotics alone or with prebiotics in coated and non-coated alginate microparticles. As prebiotics, usually FOS or isomaltooligosaccharides are used, while as coating materials, other polysaccharides or proteins. When probiotic cells were compressed into pellets and encapsulated within alginate as the coating material, significant improvement in survival (104-105-fold) was observed after exposure to acidic pH. *In vitro* tests pointed to a cell release near the end of the ileum and beginning of the colon with a mechanism involving erosion of the alginate gel layer (Eng Seng & Zhang, 2005). Many other formulations of encapsulating materials for probiotic microparticles were optimized; all of them showed improved tolerance to gastric conditions and high survival of the probiotic in colonic conditions. Of probiotics, strains of *L. acidophilus*, *L. casei*, *B. bifidum* and *B. longum*, proved to show health effects in colitis, are among the most studied. For example, *L. casei* NCDC-298 loaded Ca-alginate microparticles showed better survival of the probiotic at low pH and high bile salt concentration. In colonic pH solution, the release of cells was increased, with a count above therapeutic minimum of  $10^7$ - $10^9$  cfu g<sup>-1</sup> (Mandal et al., 2006). Similarly, encapsulated *L. acidophilus* ATCC 43121 in Ca-alginate microparticles exhibited a significantly higher resistance to artificial intestinal juice than non-encapsulated samples (Kim et al., 2008). The beads made with alginate-pectin blends provided a significant better protection to the entrapped *L. casei* under all conditions tested (Sandovall-Castilla et al., 2010). Strains of *L. acidophilus* and *L. casei* were encapsulated into uncoated Ca-alginate beads and the same beads were coated with three types of material, chitosan, Na-alginate and poly-L-lysine in combination with alginate. Chitosan-coated alginate beads provided the best protection for the lactobacillus strains in simulated GI conditions (Krasaekoopt et al., 2004). Also, chitosan coated microspheres were produced to encapsulate *L. gasseri* and *B.*

*bifidum*, separately with the prebiotic quercetin, with an aim to keep them intact during exposure to the harsh conditions of the GIT. Resistance to simulated gastric conditions during 2 h and bile salt solution for 2 h was observed (Chavarri et al., 2010).

In our study, in which the probiotic *L. casei* was microencapsulated with FOS in chitosan-Ca-alginate beads, the optimal formulation of synbiotic microparticles was stable during exposure to simulated gastric and intestinal juices and release of viable cells above the therapeutic value in the simulated colonic pH was observed (Fig. 2) (Petreska et al., 2010, 2011). Similar results were obtained when *L. casei* was entrapped in whey protein-Ca-alginate microparticles (Smilkov et al., 2011a,b). The same combination of whey protein and alginate was used for microencapsulation of strains of *L. plantarium*; only bacteria in the coated beads survived in the simulated gastric and intestinal fluid (Gbassi et al., 2009). Other protein and polysaccharides mixtures were also used to microencapsulate probiotics. For e.g, alginate-coated gelatin microspheres were prepared to encapsulate strain of *B. adolescentis*; the alginate core prevented pepsin-induced degradation of the gelatin microspheres and thus, cell release in simulated gastric juice for 2 h (Annan et al., 2008).

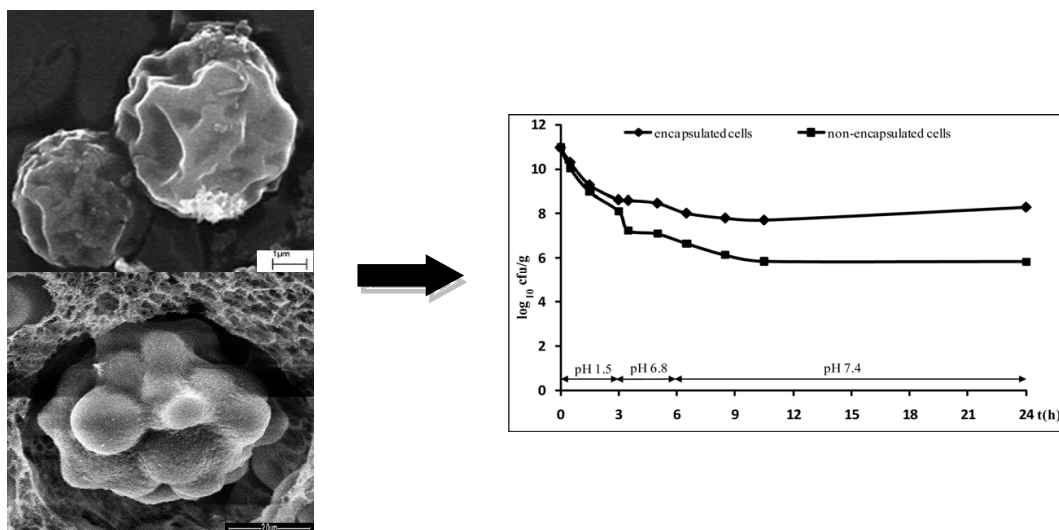


Fig. 2. Microstructure of (a) whole and (b) fractured *L. casei* loaded chitosan-Ca-alginate microparticles (left) and viability of non-encapsulated and encapsulated *L. casei* in simulated gastric conditions (0.08 M HCl; 0.2% NaCl; pH 1.5), bile salts solution (0.05 M KH<sub>2</sub>PO<sub>4</sub>; pH 6.8 with 1% bile salts) and colonic pH (0.1 M KH<sub>2</sub>PO<sub>4</sub>; pH 7.4) (right). The inner part of the particles is built of a mesh-like alginate network through which the bacteria groups are distributed and sequestered in voids.

## ii. CODES™ delivery system

CODES™ is a specific polysaccharide based system exploiting specific biodegradability of the polymers by the colonic bacteria only in combination with pH-sensitive polymer coating (Fig. 3). It is consisted of a core tablet (consisted of drug, one or more polysaccharides and other necessary excipients) coated with three layers of polymers, acid-soluble polymer (e.g., Eudragit E®) around the core, outer layer of enteric polymer and a barrier between to prevent complexation of oppositely charged polymers (e.g., HPMC) (Pantel et al., 2008). The system remains intact in the stomach. Upon entry into the colon, the polysaccharides (e.g., FOS, mannitol, lactulose, etc.) dissolve and diffuse through the coating whereby they

become subject to enzymatic degradation to organic acids. As colonic pH starts to decrease, the acidic-soluble polymer begins to dissolve, which is followed by subsequent drug release.

CODES, consisted of three components, a core containing lactulose and 5-ASA, an inner acid-soluble material layer and an outer layer of an enteric soluble material, was prepared and orally administered to fasting and fed dogs to evaluate the pharmacokinetic profiles of the drug. The results of the study confirmed that lactulose can act as a trigger for 5-ASA release in the colon (Katsuma et al., 2002). Recently, Varshosaz et al. (2011b) reported development of a novel budesonide pellets based on CODES™ technology. Pellet cores containing lactulose or manitol were coated with an acidic soluble polymer Eudragit E®100, HPMC and an enteric coat consisted of Eudragit® FS 30D. Absence of drug release in pH 1.2 and 7.4 was observed, while in medium with rat caecal contents (pH 6.8), controlled release occurred. Promising results in decreasing colitis score in animal model were also observed.

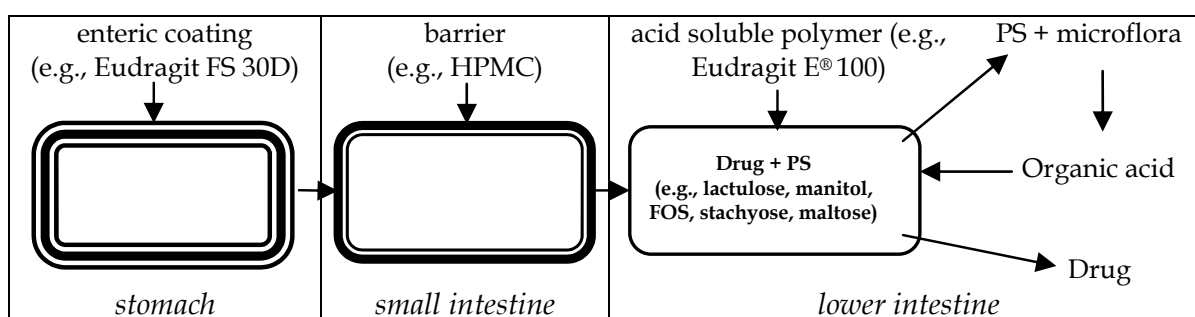


Fig. 3. Schematics of the CO(lon)DE(livery)S(ystem)™

### iii. Prodrugs

Prodrug approach for colon targeting in colitis includes formation of a covalent linkage between the drug and a carrier, which upon oral administration of the drug remains intact in the acidic environment of the stomach and upper intestine and undergoes spontaneous or enzymatic transformation in the colon. This approach solves not only the problem of achieving high drug concentration at the diseased site, but also the problem of preserving chemical stability and avoiding high systemic bioavailability and thereby toxicity. There are three classes of prodrugs commercially available or under investigation: (i) anti-inflammatory agents (e.g. 5-ASA, SCFAs); (ii) immunomodulators (e.g. corticosteroids, azathioprine); and (iii) antioxidants (e.g. glutathione, cysteine, S-adenosyl-methionine) (Chourasia & Jain, 2003; Oz & Ebersole, 2008).

All available methods for covalent linking of 5-ASA molecule were used: linking via azo-bond to another 5-ASA molecule (in olsalazine) or inert carrier 4-amino-benzoyl- $\beta$ -alanine (in balsalazine) or active carrier sulfapyridine (in sulfasalazine) and subsequent activation of the drug in the colon by the bacterial azoreductases as well as conjugation with amino acids or polymers as polymeric prodrug systems. Sulfasalazine releases 5-ASA specifically in the colon, however, a small quantity of the ingested dose is absorbed in the upper intestine resulting in serious adverse effects of sulfapyridine (Table 1). Olsalazine and balsalazine were formulated to overcome disadvantages of sulfapyridine. In the studies of Yokoe et al. (2003), a new prodrug was synthesized, salicylazosulfanyl acid. Azoreductases cleave it into 5-ASA and sulfanyl acid; owing to the high hydrophylicity of the carrier and thereby low



absorption in the GIT, adverse effects observed with sulfapyridine were avoided. The use of  $\beta$ -cyclodextrins as 5-ASA carriers in a prodrug form was characterized by a relatively successful prevention of 5-ASA release in simulated gastric and intestinal medium and subsequent release mediated by the colonic microflora (Bonsignore, 2000). Jung et al. (1998) formulated stable dextrane conjugate of 5-ASA with delayed release. Lately, they focused their research towards conjugation of 5-ASA with amino acid derivatives where 5-aminosalicyl-L-aspartic acid and 5-aminosalicyl-L-glutamic acid were synthesized and their properties as colon-specific prodrugs of 5-ASA investigated in colitic rats (Jung et al., 2001). The most of 5-ASA-Asp was delivered to the large intestine and about half of the administered dose was activated to liberate 5-ASA. Recently, an amino acid (mutual) aza-prodrug of 5-ASA was synthesized by coupling L-tryptophan with salicylic acid (Nagpal et al., 2007). *In vitro* kinetic studies showed negligible release of 5-ASA in acidic medium, while *in vivo* studies pointed to equal attenuation of the colitis in rats as that of sulfasalazine without ulcerogenicity of 5-ASA. One more attempt was made to conjugate 5-ASA for colon delivery. Specificity includes conjugation with bile acids (chenodeoxycholic and ursodeoxycholic acid) (Goto et al., 2001). *In vivo* studies in guinea pigs showed that with lower doses, higher efficacy could be achieved. Polymeric prodrugs of 5-ASA were also formulated in which 5-ASA was linked with polyacrylic or polyamide polymers via degradable ester or amide bonds (Zou et al., 2005) as well as polymeric prodrugs in which 5-ASA was bond via azo-carrier to polyanhydride polymers and derivatives of dextrane and poly[(2-hydroxyethyl)aspartamine] (Cai et al., 2003). In the recent studies of Yadav & Mahatma (2011), acrylic type polymeric systems having degradable ester bonds linked to the 5-ASA were synthesized and evaluated for colon targeted drug delivery. *In vitro* drug release studies, conducted at pH 1.2, 7.4 and in rat fecal content, pointed to a burst release of app. 40% in the first 2 h followed by a sustained release over a period of 12 h. In general, most of the mentioned prodrugs and polymeric prodrugs release 5-ASA successfully in the colon, but the complex coupling processes and the fact that the 5-ASA content is app. 10% of the total mass made them inappropriate for oral administration because a very large amount would need to be taken orally (Chourasia & Jain, 2003).

Corticosteroids were also subject to prodrug design. Steroid glycosides, galactosides and cellobiosides were designed, from which dexamethasone, prednisolone, hydrocortisone and fludrocortisone were released in the colon with hydrolysis mediated by  $\beta$ -D-galactosidase,  $\beta$ -D-glucosidase,  $\alpha$ -L-arabinofuranosidase,  $\beta$ -D-xylopyranosidase (Friend & Chang, 1985). *In vivo* researches involving rat stomach, proximal small intestine, distal small intestine and caecum pointed to the most rapidly hydrolysis in the caecal content followed by the distal small intestine. Conjugates of budesonide and dexamethasone and glucuronic acid and dextran, accordingly, were also synthesized, showing excellent efficacy in rats with induced UC and decreased toxicity, especially in respect to adrenal suppression (Nolen et al., 1997; Varshosaz et al., 2009). Also, dexamethasone 21-sulfate sodium as a colon specific prodrug of dexamethasone was prepared (Kim et al., 2006). The degree of prodrug hydrolysis and production of dexamethasone amounted to 70% of healthy rats when a prodrug was incubated with caecal contents collected from colitic rats. In comparison with prednisolone, hydrocortisone and cortisone, dexamethasone was stable against bioinactivation by the cecal contents. Anti-inflammatory effect and systemic side effects of prednisolone succinate/ $\alpha$ -cyclodextrin ester conjugate were also studied in animal model of IBD (Yano et al., 2002). The side-effects were significantly alleviated due to the passage of the conjugate through the



stomach and small intestine without significant degradation or absorption. Dextran ester prodrugs of dexamethasone and methylprednisolone, with a succinate linking the drug and dextran, were also synthesized and proved their preclinical efficacy and lower toxicity (Pang et al., 2002). Similarly, budesonide-succinate-dextran conjugate as a prodrug of budesonide showed huge improvement in macroscopic and histological scores of colitis in induced colitis in rats (Varshosaz et al., 2010). Polymeric prodrug colon delivery system of dexamethasone was also prepared, with poly-(L-aspartic acid) as a carrier with superior efficacy and lower toxicity in respect to oral dexamethasone (Leopold & Friend, 1995).

### 3.2.3 Time-dependent systems

In ideal time-controlled colon delivery system, drug release occurs after precisely determined lag phase of minimum  $3\pm 1$  h necessary for the system to pass the stomach and small intestine. In fact, the system relies on the consistent small intestine transit time, while for the formulation to withstand the individual variations in gastric emptying time and pH, usually enteric coating is used. This prevents rapid swelling and disintegration in the upper GIT, while in the colon, drug release rate is controlled by the mechanisms of swelling, osmosis or diffusion, erosion or a combination of all (Singh, 2007). In general, it is very difficult colon specific and controlled release in a state of colitis and diarrhea with this type of delivery systems to achieve because the transit through different regions of the colon is accelerated and unpredictable.

Various structures and formulation designs were commercialized (Pulsincap<sup>®</sup>, Time Clock<sup>®</sup>) and described for this type of delivery systems, mostly adapted from the pulsatile delivery systems. They can be subdivided into reservoir and capsular formulations prepared in a form of single- or multiple-unit preparations (Iamartino et al., 1992; Takada, 1997; Ueda et al., 1989). Usually, in all designs, drug, one or more swellable hydrophilic excipients (e.g., sodium starch glycolate, CMC sodium, low substituted HPC) and water-insoluble enteric polymers (e.g., EC, Eudragit<sup>®</sup> RL) are present. Patent assigned to Hoffman-La Roche (Shah et al., 2000) contains also a plasticizer in an inner semi-permeable polymer membrane, which allows water influx but prevents the outward diffusion of the drug. An outer enteric-coating, which dissolves above pH 5.5, swells during the transit of the tablet through the small intestine and after a consistent period of minimum 4 h transit in the small intestine, the swollen core bursts the semi-permeable membrane and the drug is released in the colon. In the erodible systems, the penetration of the GI fluids through the micropores of the outer layer causes swelling/expansion, dissolution and/or erosion of the swelling agent(s), which accordingly, pushes the drug out of the system or delays the drug release for a time determined with the selection of the coating polymer(s). In rupturable reservoirs, a time for release of the drug is programmed by the disruption of a semi-permeable membrane consisted of insoluble polymer(s). In the device of Ritschel & Agrawal (2002), for example, self-destruction of the semi-permeable membrane occurs and the drug goes out of the system through the orifice made by a specific press-coating. Concerning the capsular systems, the drug release occurs after dissolution of a protective polymer cap and subsequent removal of a matrix plug from a drug-containing insoluble capsule body; the time of ejection corresponds to the lag phase.

Time-dependent system for delivering 5-ASA to the colon was prepared in which the core tablet of 5-ASA was compression-coated with HPMC and then coated with Eudragit<sup>®</sup>L-100.

The results revealed that the lag time increases with the amount of HPMC (Patel et al., 2009). Combined time- and pH-dependent microparticulate system consisting of non-enzymatically degrading PLGA core for delivering budesonide specifically to the distal ileum and colon was also developed. Eudragit®S-100 was used to form a coating on the surface of the microparticles. Complete retardation of drug release in an acidic pH and controlled release in pH 7.4 and 6.8 was observed (Krishnamachar et al., 2007). In addition, Yehia et al. (2009) optimized a formulation of budesonide loaded compression-coated tablets where as time-dependent variable, cellulose acetate butyrate was used.

### 3.2.4 Osmotic-controlled systems

In general, in osmotic drug delivery systems, the delivery of the active agent(s) is delayed or pulsed, driven by an osmotic gradient and it is not dependent /affected by the physiological variables within the GIT. After administration, the water diffuses into the core of the osmotic system through a semi-permeable membrane increasing the hydrostatic pressure, which pumps the active agent containing solution out of the core through one or more orifices. The drug release follows zero order kinetics and the rate is controlled by the diffusion rate of the water into the system (Gupta et al., 2009).

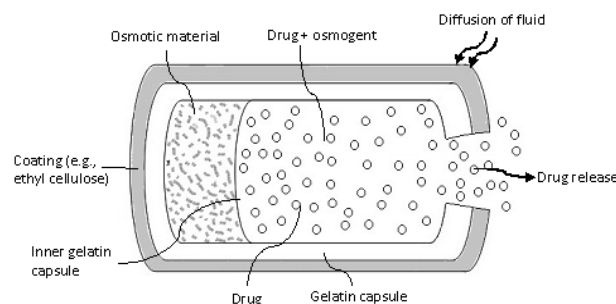


Fig. 4. Osmotic pump capsule structure

Lots of osmotic delivery systems, with membrane plug retention mechanism or with osmotic device in an osmotic device, have been patented, with a potential to carry the drugs for the treatment of different states of colitis. Therapeutic System Research Laboratory Arm Arbor (Michigan, USA) developed the Port system consisting of a capsule coated with a semi-permeable membrane. Inside the capsule is an insoluble plug consisting of osmotically active agent and the drug formulation (Fig. 4). Osmotic system OROS-CT was designed by Alza Corporation to target the active agent(s) to the colon. It is a system composed of a single or multiple (5-6) bi-layered osmotic units encapsulated within a hard gelatin capsule. Each layer, the push and drug layer, are surrounded by a semi-permeable membrane, while the orifice is drilled through the membrane next to the drug layer. Semi-permeable membrane, usually consisted of CA and cellulose acylate, is insoluble in body fluids, non-erodible, but permeable to the passage of fluids. Each unit is surrounded by enteric coating, usually phthalates, keratin, formalin-treated protein, oils and anionic polymers, which do not dissolve, disintegrate or change their structure in the stomach. The osmogents are either hydrophilic polymers (e.g., HPMC, poly(hydroxyalkyl-methacrylate), poly(vinyl-pyrrolidone), poly(vinyl alcohol), acidic carboxy-polymers or inorganic water-soluble agents (e.g., magnesium sulfate, sodium- and potassium chloride, sodium bicarbonate, etc.). In UC,

drug release begins when the drug enters the colon, with 3-4 h post gastric delay. Then, a constant release follows which may last up to 24 h (Gupta et al., 2009; Patel et al., 2010).

Specifically, a new microbial-triggered colon targeted osmotic pump was developed for colon delivery of budesonide based on chitosan. Chitosan was used to produce osmotic pressure by swelling and with its degradation, *in situ* delivery pores were formed through which budesonide was released. CA along with chitosan was coated on a tablet as a semi-permeable membrane, while as entering coat, Eudragit®L-100-55 was used (Liu et al.; 2007).

### 3.2.5 Pressure-controlled systems

The rationale behind the design of the pressure-dependent colon delivery systems lies in the existence of strong peristaltic waves that move intestinal content from ascending to transverse colon, temporarily increasing the luminal pressure within the colon. Significantly higher viscosity of the colonic content is a reason for much higher luminal pressure in the colon in comparison with the one in the small intestine. So, the delivery system is formulated to withstand the pressure in the upper GIT and to collapse in the lower intestine.

So far, pressure-controlled colon delivery capsules (PCDCs) as a unique system were prepared by coating an inner surface of gelatin capsules with EC. By adjusting the coating thickness of the EC membrane, colon delivery of 5-ASA in beagle dogs was obtained. Namely, after administration, 5-ASA appeared into the systemic circulation after 3-5 h, which corresponds to the colon arrival time observed with sulfasalazine (Muraoka et al., 1998). Avoiding side effects of sulfapyridine is a great achievement with this type of colon delivery system. Furthermore, 5-ASA was loaded in microcapsules prepared of EC or Eudragit L-100 or S-100 and filled into PCDCs, which were prepared as fast release colon delivery system with 5-ASA powder suspended in a suppository base. The release rate of 5-ASA from the microcapsules was significantly prolonged as compared to 5-ASA powder with no significant differences in the release rates between the microcapsules. The first appearance time of 5-ASA into the systemic circulation after oral administration was 3 h for all the colon delivery preparations, while both EC microcapsules and Eudragit®S-100/RS-100 microcapsules in PCDCs showed longer mean residence time than Eudragit®L-100/RS-100 microcapsules, suggesting sustained release characteristics (Hu et al., 1999).

## 4. Conclusion and future perspectives

Undoubtedly, advanced colon drug delivery systems (CDDSs) offer significant advantages in respect to both efficacy and safety of drugs used for the treatment of colitis and considering probiotic cells, significantly increased survival and colonization rate. However, commercial products for oral administration based on the mentioned CDDSs for all the drugs needed for the treatment of colitis, especially for biologic drugs, are still not available. In this respect, multidisciplinary project was initiated by the research group from the University Medical Center Groningen to develop and evaluate oral formulations of infliximab, including formulation based on pH-responsive coating containing Eudragit®S-100. Similarly, formulation of infliximab loaded PLGA microspheres was developed by Foong et al. (2010) as a prospective novel treatment of CD fistulae showing controlled release under zero-order kinetics of the anti-TNF- $\alpha$  antibody and biological activity against

TNF- $\alpha$ . Also, to the present knowledge, no commercial pharmaceutical product containing microencapsulated probiotic cells exists.

Of all the primary above-mentioned approaches proposed for the CDDSs none is ideal when separately used and colon specificity and controlled delivery is more likely to be achieved with systems based on mucoadhesive natural materials that are degraded by the colonic bacterial enzymes. Considering the complexity of the CDDSs and the difficulties in establishing *in-vitro/in-vivo* correlation by actual dissolution methods, a validated dissolution method for their evaluation has to be developed, which considers the physiological characteristics of the colon and can be used routinely in an industry. In addition, novel approaches developed for colon-targeting and controlled release, as even more specific, have to be comprehensively explored and commercialized as drug/cell carriers in the treatment of colitis. Extensive clinical data showing promising efficacy in active colonic diseases, including IBD, are now available for TARGIT Technology (West Pharmaceutical services) designed for targeted release into the colonic region. The technology is based on application of pH-sensitive coatings onto injection-moulded starch capsules. Also, the ENTERION capsule has recently been developed (Phacton Research, UK) for targeted delivery of different drug formulations into any region of the gut. The rounded capsule sealed by inserting a push-on cap fitted with a silicone O-ring can be loaded with either a liquid formulation or a particulate formulation (e.g., micro/nanoparticles, pellets, etc.). The bottom of the drug reservoir is the piston face which is held back against a compressed spring by a high tensile strength polymer filament. Once the capsule reaches the target location in the GIT, the drug is actively ejected by the external application of an oscillating magnetic field. Clinical application and converting this tool into a product-carrier of drugs for colitis treatment remains a big challenge. Therefore, a search for new delivery systems that can provide increased therapeutic benefits to the patients with colitis continues.

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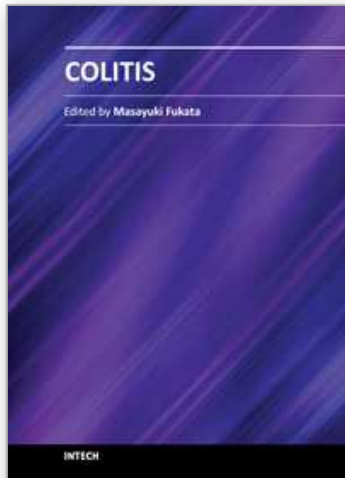


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Edited by Dr Fukata

ISBN 978-953-307-799-4

Hard cover, 196 pages

**Publisher** InTech

**Published online** 05, January, 2012

**Published in print edition** January, 2012

Inflammation of the colon is collectively called "Colitis". Since a variety of conditions may cause colitis and its manifestations are similar among the causes, selection of the right treatment based on the correct diagnosis is important in the management of this group of illnesses. Over the last few decades, a major shift has been observed in the clinical attention to the pathogenesis of colitis from infectious to idiopathic inflammatory bowel diseases. Colitis cases that are associated with chemical therapeutics and specific pathogens such as amoeba, have become prominent in hospitalized individuals and immune deficient patients, respectively. In addition, a great deal of progress has been made in colitis research triggering the need for updating our knowledge about colitis. This book Colitis provides comprehensive information on the pathogenesis, mechanism of resolution, and treatment strategies of colitis.

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