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Review

Advances in the colon-targeted chitosan based multiunit drug delivery systems for the treatment of inflammatory bowel disease

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Starch (24836924)
Xanthan gum (131750926)
Pectin (5381226)
Carboxymethyl cellulose sodium (23706213)
Dextran sulphate (4125253)
5-Aminosalicylic acid (4075)
Eudragit S-100 (65358)
Sodium tripolyphosphate (24455)

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ABSTRACT

Chitosan is the polymer of choice for delivery of the active moieties to the colon due to its cationic nature that enables strong mucosal attachment. Chitosan is explored for formulations such as pellets, beads, microspheres, nanoparticles and drug-polymer conjugates for colon targeting of various therapeutic agents in inflammatory bowel disease (IBD). The major challenge in the colonic delivery of drugs in IBD is altered physiological pH, which can be addressed *via* chitosan containing multiparticulate drug delivery systems owing to their biodegradability in the colon. Its ionic interaction with anionic polymers forms gastro-resistant multi-unit systems that ensures safe delivery of payloads to the colon. In contrast to commercial grade gastro-resistant polymers, chitosan has GRAS (generally regarded as safe) status that ensures safety for long-term therapy in case of chronic diseases such as IBD. Here, we review in detail essential properties of chitosan and chitosan based multiunit formulations for treatment/mitigation of IBD.

Abbreviations: APIs, active pharmaceutical ingredients; GRAS, generally regarded as safe; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; 5-ASA, 5-amino salicylic acid, mesalamine; LD_{50}, lethal dose; GIT, gastrointestinal tract; EC, ethylcellulose; ROS, reactive oxygen species; TLRs, Toll-like receptors 4; NODs, Nod-like receptors; LPS, lipopolysaccharides; NF-κB, nuclear factor kappa B; MAPK, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase; JNK, C-JUN-N-terminal kinase; DCs, dendritic cells; COS, chitosan oligosaccharides; TNF-α, tumor necrosis factor-α; IL, interleukins; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; ($PG-E_2$), prostaglandin E_2; NO, nitric oxide; PF, platelet factor; MCC, microcrystalline cellulose; SIF, simulated intestinal fluid; EDTA, ethylenediaminetetracetic acid; CHI-g-AAm, acrylamide grafted chitosan; MPO, myeloperoxidase; TNBS, 2,4,6-trinitrobenzenesulfonic acid; M, mannuronic acid; G, guluronic acid; CAB, cellulose acetate butyrate; DSS, dextran sulphate; PCR, polymerase chain reaction; MyD 88, myeloid differentiation primary response 88; GMPs, glucan mannan particles; HNT, halloysite nanotubes; LMWH, low molecular weight heparin; TMC, trimethyl chitosan; KPV, Lys-Pro-Val; Map4k4, mitogen-activated protein kinase kinase kinase kinase 4; MGL, macrophage galactose-type lectin; PLGA, poly lactic-co-glycolic acid; US-FDA, US-Food and Drug Administration; CMC, carboxymethyl chitosan; 6-MP, 6-mercaptopurine; GSH, glutathione; CH-EDTA, chitosan-ethylenediaminetetracetic acid; I.V., intravenous; S.C., subcutaneous; aPTT, ATPP, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; CRC, colorectal cancer; BBE, Brush border enterocytes; mRNA, messenger RNA; IRF, interferon regulatory factor; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; VEGF, vascular endothelial growth factor.

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Inflammatory bowel disease
Colon targeting
Sodium alginate
Biopolymers
Multiparticulate drug delivery

1. Introduction

1.1. Inflammatory bowel disease and its current status worldwide

The term IBD represents two clinically defined conditions known as Crohn's disease (CD) and ulcerative colitis (UC), which are characterized by chronic inflammation of the gastrointestinal tract, collectively affecting 6.8 million population worldwide in the year 2017 (Alatab et al., 2020; Shah, Palakurthi, Khare, Khare, & Palakurthi, 2020). The mortality rate of IBD is 40% as compared to those without evidence of the disease (Oz & Ebersole, 2008). Despite the large population suffering from IBD, the exact etiology of the disease is not yet completely understood, however, extrinsic factors such as microorganisms and chemical compounds or autoimmune disease are known contributors. Other factors reported to have role in the causation of the disease are genetics, unhygienic conditions, immune dysfunction, environmental factors such as diet, stress, pollution, cigarette smoking, pathogenic infections and microbiome imbalance (Hua, Marks, Schneider, & Keely, 2015a; Zhang, Langer, & Traverso, 2017). As shown in Fig. 1, CD usually affects terminal ileum and the colon in the discontinuous pattern of mucosal inflammation, on the other hand UC limits itself to the large intestine and may extend to rectum or entire colon with continuous pattern of mucosal inflammation. IBD manifests clinically in the form of abdominal pain, weight loss, vomiting, fever, or bloody diarrhea. IBD have been associated with extra-intestinal manifestations in the form of arthritis, sacroiliitis, and ankylosing spondylitis (Langhorst, 2009). There are a number of factors associated with the disease that affect a patient's quality of life significantly, *viz.* early onset, no permanent cure, and frequent flare-ups; all these factors lead to severe morbidity among the patients. Previously, the disease was considered to be confined to the western/developed world, but in the last decade or two there has been significant rise in the number of cases reported in Asian countries, which can also be attributed to the availability of advanced detection techniques, such as endoscopy and colonoscopy, which were not available earlier, especially during 80's. The rise in the incidence of the disease is also correlated to industrialization which has started in the western world and is now growing significantly in the Asian countries (Das et al., 2009; Kedia & Ahuja, 2017).

As there is no permanent cure of the disease, therapy options are mainly aimed at maintenance of remission and prevention of flare-ups of inflammation. If untreated, IBD exacerbate itself in the form of clinical or paraclinical symptoms such as rectal bleeding, anemia, gastrointestinal (GI) spasm, nausea, fever, fatigue, loss of weight and loss of appetite. These symptoms form a base for classification of the disease into three stages as mild, moderate or severe. Treatment options for IBD includes 5-amino salicylic acid (5-ASA) (an anti-inflammatory agent (for mild IBD), corticosteroids such as prednisolone, budesonide, beclomethasone dipropionate, *etc.* (for moderate stage), immunosuppressive agents, like methotrexate, azathioprine, 6-mercaptopurine (6-MP), cyclosporin-A, *etc.* and anti-TNF-α-antibodies, like infliximab, adalimumab, certolizaumab, *etc.* for the severe stage of the disease (Lautenschläger, Schmidt, Fischer, & Stallmach, 2014; Rawla, Sunkara, & Raj, 2018; Talaei, Atyabi, Azhdarzadeh, Dinarvand, & Saadatzadeh, 2013; Zhang & Merlin, 2018).

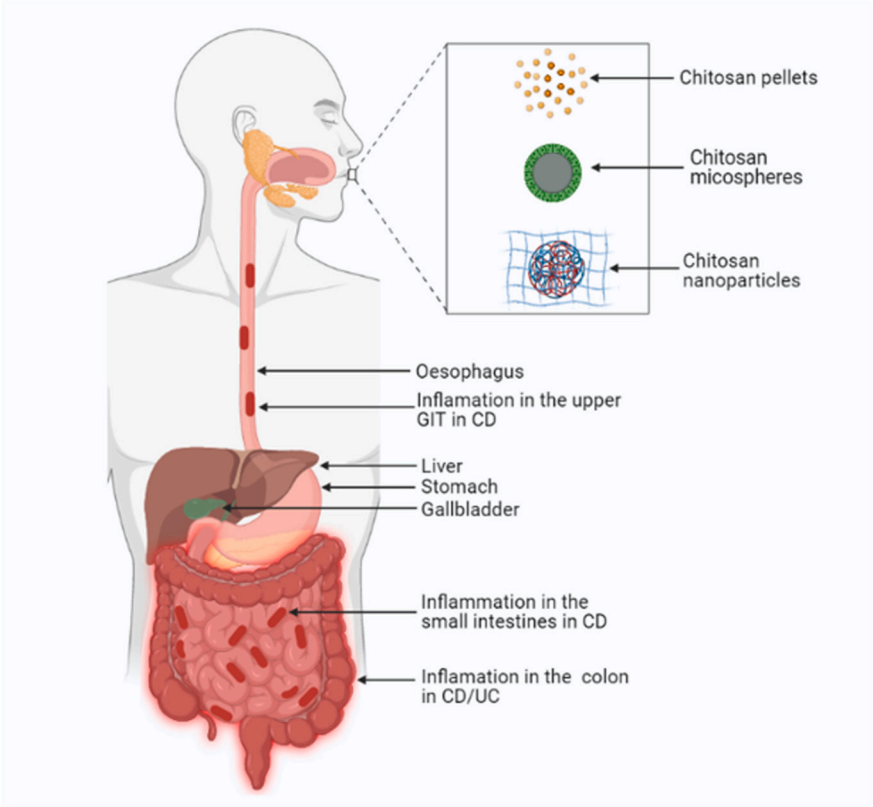

Fig. 1. Term IBD encompasses both the conditions, Crohn's disease (CD) and ulcerative colitis (UC), while UC and CD share many clinical features, UC remains confined to terminal ileum and the colon, on the other hand CD can affect any region of the GIT. The other differentiating point is depth of inflammation. UC could affect up to the innermost mucosal layer, and CD penetrates the deeper portions instead of being confined to the mucosal layers. Chitosan based drug delivery systems have the potential of targeting the payloads to the colon.

Table 1
Comparison of normal and altered physiology of the gastrointestinal tract in IBD.

Gastrointestinal organs	Normal luminal pH	pH in active IBD	Normal GI transit time (h)	GI Transit time in IBD (h)	References
Stomach	1.2–2 (fasted) 2–6 (fed)	~2	2–3	N/A	(Hua, Marks, Schneider, & Keely, 2015b; Nugent, Kumar, Rampton, & Evans, 2001; Zeeshan, Ali, Khan, Khan, & Weigmann, 2019)
Proximal small intestine	5.5–7.0	6.1–7.3	4–6	Prolonged	
Distal small intestine	6.5–7.5	7.2–8.3			
Caecum/right colon	5.5–7.5	2.3–7.2	41.1–62.3	9.5–39.1	
Left colon/ rectum	6.5–7.5	5.3–7.5			

The oral route for drug administration is the preferred one because of ease of administration, patient compliance, cost effectiveness, and ease to cut-off absorption of a drug at any time point. However, due to physiology of the GI tract, a dosage form exposes to different environmental conditions at different gastrointestinal sites, such as acidic pH in the stomach, slightly acidic to neutral in the small intestine and slightly basic in the large intestine. Besides, there is also a large variation in the gastrointestinal transit time post meals. Alteration in the gastrointestinal physiology in IBD patients is described in Table 1. All the above-mentioned factors collectively pose a serious challenge for the formulation scientists to target a drug to the colonic site *via* oral administration.

The commonly used formulation strategies for drug targeting to the colon include time-dependent release, pH responsive polymeric coating, pro-drug approach, colonic microbiota initiated drug delivery, conjugation of a drug with a polymer/biopolymer, bioadhesive drug delivery and osmotically controlled drug delivery (Cesar et al., 2018; Chourasia & Jain, 2003; Kotla et al., 2019; Sinha & Kumria, 2003; Vass et al., 2019).

2. General properties of chitosan in view of the colon targeting

2.1. Chemistry of chitosan

Chitosan, synthesized from chitin, is a polysaccharide with exceptional physical and biological properties. Chitin is generally present in the shells of crustaceans and shrimps and fungal cell walls. Chitin was introduced to the world in the year 1884, which is the second most abundant polysaccharide in nature after cellulose (Zeeshan et al., 2019). Chitosan is obtained from chitin by deacetylation, which is only naturally occurring cationic polymer. It consists of β-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose (Fig. 2). Chemically, chitosan is a linear amino-polysaccharide chain comprising of randomly linked-β (1→4) linked D-glucosamine and *N*-acetyl D-glucosamine units arranged in a random fashion. Elemental analysis demonstrated that chitosan possesses greater than 7% nitrogen content and less than 0.04% degree of acetylation. Normally, the commercial chitosan has 60–100% degree of deacetylation and its molecular weight ranges between 20 and 1200 kDa. Synthesis of chitosan from chitin requires harsh conditions needed for removal of acetyl groups using concentrated sodium hydroxide solutions; this is an issue warrants economic as well as ecological problems. Therefore, techniques have been sought to design synthetic methods that would employ less amount of sodium hydroxide solution (Nugent et al., 2001).

The presence of deactylated primary amine group in chitosan is important to elicit the ability to undergo desired modifications for the site-specific drug delivery. Chitosan is a weak base with p*Ka* values ranging from 6.2 to 7.0, therefore, it is insoluble at neutral and alkaline pH. In acidic conditions, the amine groups of the polymer undergoes protonation followed by solubilization, resulting in the positively charged polysaccharide with high charge density. This positively charged polymer has ability to interact with the GI mucosa, which is essential for long residence time at the site, desirable in case of IBD, as diarrhea is very common symptom of the disease. Apparently, chitosan is degraded by hydrolysis in humans primarily by enzyme lysozyme and bacterial enzymes in the colon such as Chitinases secreted by the Bacteroids. Looking at the physiology of the GIT, due to the acidic pH in the stomach, concentration of bacteria is very low, that gradually increases along small intestine and there is significant rise in their number in the colon probably due to favorable pH. Therefore, chances of chitosan being metabolized in the upper GIT are very rare. Other factor that controls rate and extent of degradation of chitosan is its degree of deacetylation, higher it is less is its degradation. Thus, degradation by colonic microflora makes chitosan a potential polymer for colon specific drug delivery (Hejazi & Amiji, 2003a, b; Kalantari, Afifi, Jahangirian, & Webster, 2019b; Kean & Thanou, 2010; Ray, 2019). All these properties possessed by chitosan makes it very special, displaying advanced physiochemical properties that are explored for biomedical applications. Polymer chain length and varying acetyl group distribution are important governing factors for the biodegradation kinetics and sustained release of the drugs which may prove important for management of IBD (Kalantari et al., 2019a, b).

2.2. Biocompatibility and biodegradability

Owing to the semi-crystalline nature, chitosan is insoluble in aqueous solutions above pH 7; however, it is freely soluble below ~pH 5 due to protonation of the amino group present on it. Chitin is also semi-crystalline, with intermediate level of degree of deacetylation that imparts minimum crystallinity (Chourasia & Jain, 2003; Vass et al., 2019). Chitosan metabolizes within the human body *via* hydrolytic cleavage of the glycosidic bond joining polysaccharide units in the polymer. This degradation converts the polymer into glucosamine and saccharide units. Apparently, degradation kinetics is dependent upon the degree of crystallinity and degree of acetylation of the polymer, as the latter regulates the former, greater the acetylation the more crystalline the polymer (Chourasia & Jain, 2003; Francis Suh & Matthew, 2000;

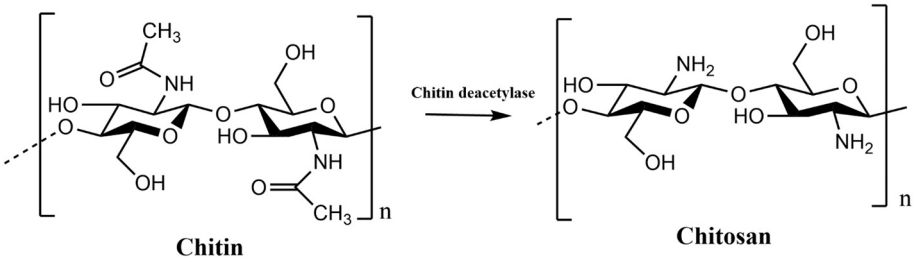

Fig. 2. Synthesis of chitosan from chitin.

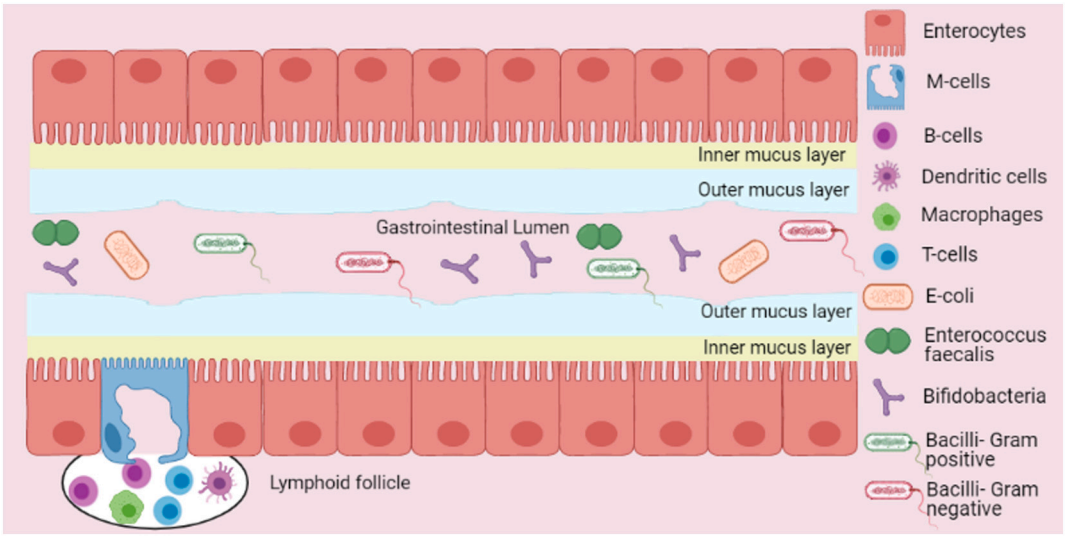

Fig. 3. Healthy colonic mucosa has closely packed enterocytes over which inner and outer layers of mucus are present; these two features jointly prevent entry of foreign materials and luminal contents, but absorption of nutrients. Here, commensal microbiota and immune system are in harmony with each other. Out of the number of components responsible for IBD, imbalance of microbiota (dysbiosis) is one. M-cells lying in lymphoid tissues initiate immune response. Various cells responsible for the immune responses in gastrointestinal lumen reside in the lymphoid follicles.

Kalantari, Afifi, Jahangirian, & Webster, 2019a; Lorenzo-Lamosa, Remuñán-López, Vila-Jato, & Alonso, 1998; Vass et al., 2019). Another distinctive attribute of chitosan which contributes towards its biocompatibility is its low LD_{50} value, which is reported to be greater than 16 g/kg in mice (Dodane & Vilivalam, 1998). In addition to the above mentioned features another advantage of chitosan is its degradation by colonic microflora which enables its exploration for colon specific drug delivery in IBD (Hejazi and Amiji, 2003a, b). Mcconnell et al. tried to answer the fundamental question, does chitosan metabolize by the enzymes present in the colon? They used human faecal material and porcine pancreatic enzymes in the study. Authors concluded that colonic microflora metabolizes chitosan which is a function of its cross-linking. Non cross-linked films of chitosan were metabolized within 4 h, however when cross-linked by using glutaraldehyde and sodium tripolyphosphate, it resisted digestion over 4 h. Further, sodium tripolyphosphate cross-linked films resisted the metabolism by pancreatic enzymes for up to 18 h (Mcconnell, Murdan, & Basit, 2008). Tokazi et al. reported chitosan capsules for colonic delivery of insulin. Authors coated the capsules by an enteric polymer to protect it from the harsh acidic environment in the stomach; outcomes of the study revealed chitosan

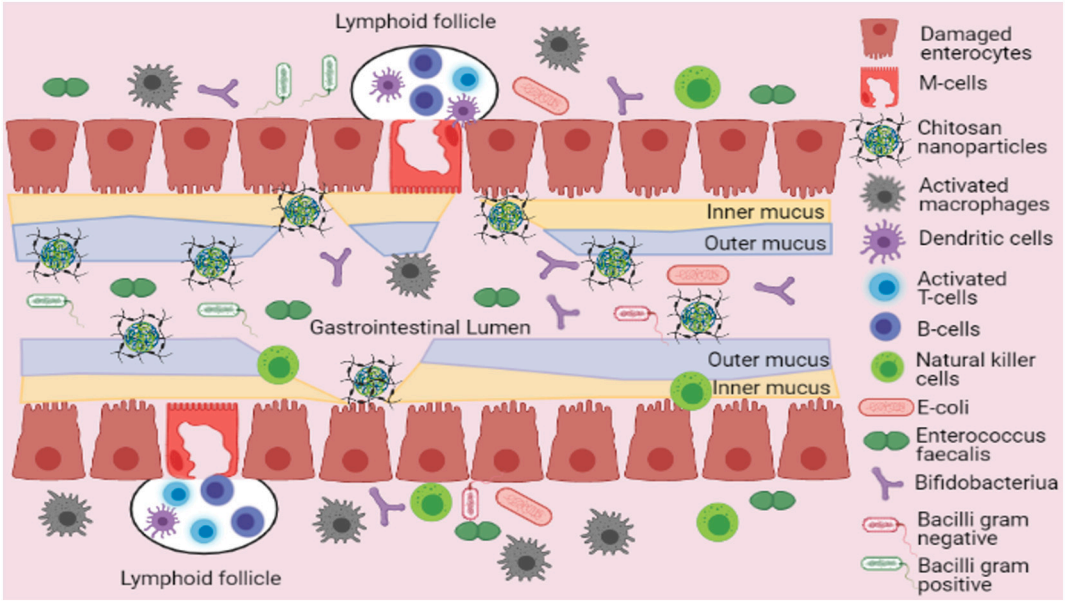

Fig. 4. Inflamed colonic mucosa has distorted morphology of enterocytes, due to erosion of the microstructures present on their surface there is loss of selective permeability of the cells. There is also disruption of the inner and outer layers of mucus. External stimuli triggers response initiated by the M-cells, cells involved in innate immunity-macrophages, dendritic cells, and natural killer cells and cells involved in adaptive immunity-lymphocytes such as B-cells and T-cells called at the site of stimuli. Chemokines such as IL-8, platelet factor-4 (PF-4) produced by activated lymphocytes play an important role as chemoattractants. Balanced microbiota not only facilitates differentiation of naïve gut DCs into tolerogenic DCs but also generate regulatory T cells thereby establishing immune homeostasis. The harmony between intestinal mucosa and microbiota is lost in severe inflammation that leads to increased infiltration across epithelial barriers by the intestinal bacteria. Chitosan nanoparticles interact with the anionic sialic acid groups of the mucus that improves their retention time in the inflamed colon, where of the several clinical manifestations of IBD, diarrhea is predominant.

capsules were degraded by proteolytic enzymes present in rat cecal content (Tozaki et al., 1997).

2.3. Delayed and controlled/sustained release of drugs

Delayed release has significance in terms of site-specific drug release in the gastrointestinal tract (GIT), which offers number of advantages, such as, 1. No unwanted distribution of the drugs in the body, 2. Possible reduction of the dose, and 3. Achieves maximum concentration of the drugs at the desired site. Delayed release of an API can be achieved through various formulation approaches using chitosan that are discussed in subsequent sections in detail. Sustained/Controlled release of the drugs ensures prolonged action at the site, which is essential for mitigation of chronic inflammation in IBD. Multiparticulate dosage forms such as nano- or microparticles are especially important in achieving sustained as well as site-specific drug release. Because of their small size, they are taken-up easily by the cells involved, and if made-up of chitosan, its mucoadhesive properties ensures prolonged stay at the site, which is otherwise difficult using available/marketed polymers in case of inflammatory bowel disease due to severe diarrhea. In addition to that, chitosan can reversibly open tight junctions between the epithelial cells and promotes paracellular transport of the encapsulated drugs (Du, Liu, Yang, & Zhai, 2015).

In case of chitosan its crystallinity and molecular weight is important to regulate its dissolution at the acidic pH of the stomach. Crystallinity can be controlled by degree of deacetylation, lesser is the degree of deacetylation more crystalline is chitosan. A new dimension in the controlled/sustained release of payloads has been introduced since the biodegradable (natural and synthetic) polymers are employed for the purpose. Encapsulated drug materials show slow and controlled diffusion through these polymeric membranes/matrices. In another mechanism of controlling drug release using chitosan, drugs are covalently attached to the polymer or they are dispersed into its matrix, its biodegradation/erosion would facilitate the release. Chitosan also has gel-forming ability at low pH that may provide rate-controlling barrier (Ravi Kumar, 2000). Multilayer coatings using natural polymers by exploring opposite charge present on their surface is also a suggested approach for controlled release applications (N. Mengatto, Helbling, & Luna, 2012).

Chitosan acetate, a derivative of chitosan along with ethylcellulose (EC) was explored for pH-, time-, and enzyme- controlled release of the model drug 5-ASA in the compressed coated tablet formulation (Nunthanid et al., 2009). Besides, various research groups has reported controlled/sustained release applications of chitosan in the form of multi-particulate formulations (Bharathala, Singh, & Sharma, 2020; Murali et al., 2020).

2.4. Anti-inflammatory effect of chitosan in IBD

Inflammations are the protective biological reactions that intend to protect a human body from the harmful stimuli. These stimuli can be triggered by infectious agents such as virus or bacteria or their components, physical agents, reactive oxygen species (ROS), hypoxia, to name a few (Chovatiya & Medzhitov, 2014). In certain situations, inflammatory reactions may go dysregulated and cause acute or chronic inflammation that leads to tissue or organ damage. Heathy *vs* inflamed mucosa is depicted in Figs. 3 and 4, respectively. Microbial components are one of the contributory factors to the etiopathology of IBD. In a series of events that lead to inflammation in IBD, firstly, the pattern recognition proteins of toll-like receptors 4 (TLRs) and nod-like receptors (NODs) recognize pathogen-associated molecular patterns associated with bacterial components such as lipopolysaccharides (LPS) which then initiates innate immune response by degradation of IκB which allows translocation of nuclear factor kappa B (NF-κB) into the nucleus of macrophages. NF-κB accounts for the regulation of pro-inflammatory mediators responsible for the inflammation. Another pathway that initiates immune responses is mitogen-activated protein kinase (MAPK)-dependent pathways (Muanprasat & Chatsudthipong, 2017; Ngo et al., 2015; Tu, Xu, Xu, Ling, & Cai, 2016). MAPK has three distinct downstream mediators: extracellular-signal-regulated kinase (ERK), P 38 MAPK, and C-JUN-N-Terminal kinase (JNK).

Innate immune response acts as a frontline defense system against stimuli and in case of IBD it is microbial and environmentally borne antigens. This immune response is non-specific and does not grant long-lasting immunity. It is mediated by a variety of cells that includes typical immune cells such as neutrophils, monocytes, dendritic cells (DCs), macrophages and non-immune cells such as intestinal epithelial cells, endothelial cells, and myofibroblasts (Fig. 4). Adaptive immune response is a result of inability of acute immune response in clearing antigenic materials due to defective autophagy and recognition of microbial and inflammatory debris. Adaptive immune response is highly specific, and provides long lasting immunity. T-cells are the important mediators of the adaptive immune response. Dysregulated innate and adaptive immune pathways contribute towards intestinal inflammation in IBD (Dave, Papadakis, & Faubion, 2014; de Souza & Fiocchi, 2015; Geremia, Biancheri, Allan, Corazza, & Di Sabatino, 2014).

Chitosan oligosaccharides (COS) exhibit anti-inflammatory activity by inhibiting responses initiated by macrophages that are induced by microbial debris. Large amount of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα), IL-6, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), prostaglandin E_2 (PG-E_2), and nitric oxide (NO) produced by macrophages contribute significantly to the pathogenesis, onset and progression of UC; COS has been shown to inhibit expression and release of these pro-inflammatory mediators. Mechanisms involve downregulation of JNK ½, prevention of phosphorylation of p38 MAPK, and IκB degranulation (Etzerodt et al., 2012; Muanprasat & Chatsudthipong, 2017; Song et al., 2016). Intestinal barrier dysfunction is another clinical manifestation of IBD. Very viscous inner layer and less viscous outer layer of mucus forms a formidable intestinal barrier that prevents translocation of pathogens across the epithelial layer. Partial or complete erosion of the mucus layers and disruption of the tight junctions between epithelial cells allow entry of harmful pathogens. COS has been reported to improve integrity of the intestinal epithelial barrier by promoting tight junction assembly (Chovatiya & Medzhitov, 2014; Muanprasat et al., 2015; Yousef, Pichyangkura, Soodvilai, Chatsudthipong, & Muanprasat, 2012). In the first of its kind of study, Wang and research group has reported intestinal mucus modulating activity of COS on human colonic mucus secreting HT-29 cells (Wang, Wen, et al., 2021). In a histological event infiltration of innate immune cells (macrophages, neutrophils, dendritic cells, and natural killer cells), and adaptive immune cells (lymphocytes such as T and B cells) takes place into the lamina. Neutrophils, basophils are the granulocytes; they actively participate in inflammation by secreting pro-inflammatory cytokines. Neutrophils, earlier thought to be brave warriors fighting against bacterial infiltration, over the time emerged as major damage causing cells that worsen inflammation by releasing ROS, proteinases, and cationic peptides (Zhang, Jiang, et al., 2020). COS obstruct activation of basophils, neutrophils and lymphocytes. Moreover, COS prevents histamine release and thereby production of pro-inflammatory cytokines such as IL-1β, IL-4, IL-6, IL-8 and IL-13, in basophils, by suppressing the calcium-induced activation of MAPK signaling pathways that include ERK1/2, and p38 (Muanprasat & Chatsudthipong, 2017). Oxidative stress mediated *via* ROS such as superoxide radicals, hydroxyl radicals, peroxyl, alcoxyl, and hydroperoxyl has significant role in worsening of IBD in term of damaging mucosal lining and bacterial invasion (Tian, Wang, & Zhang, 2017). COS is reported to attenuate oxidative stress induced apoptosis in human colonic epithelial cells (T84 cells) (Yousef et al., 2012).

Controlling inflammation and achieving mucosal healing are the main goals of UC treatment (Iacucci, De Silva, & Ghosh, 2010; Pineton De Chambrun, Peyrin-Biroulet, Lémann, & Colombel, 2010), multiple drug combination therapy has been proposed as a potential strategy

(Lee, Gangireddy, Khurana, & Rao, 2014; Ni et al., 2016). For restoration of intestinal homeostasis, there have been numerous studies reported benefits of a pro-healing cytokine, Interleukin-22 (IL-22) as: (1). It facilitates the proliferation, survival and reconstitution of epithelial cells, avoiding microbiota from further penetrating into the colonic tissues (Ouyang, 2010; Zindl et al., 2013), (2). It enhances production of mucus-associated proteins and induces regeneration of goblet cells, that lead to the formation of essential static external barrier which separates intestinal flora from intestinal epithelial cells (Sugimoto et al., 2008), and (3). It stimulates secretion of a large amount of antimicrobial peptides through expression of intestinal epithelial cells and Paneth cells, which kill invading or sequester pathogens (Sugimoto et al., 2008; Zenewicz et al., 2008). It is reported in some preclinical studies based on wild-type mice or IL-22-deficient mice subjected to dextran sulphate sodium (DSS)-induced UC, treatment with a IL-22-neutralizing antibody augmented damage in the colonic epithelial layer, induced severe weight loss and increased inflammation in the colon (Neufert et al., 2010; Pickert et al., 2009). In the research work reported by Sugimoto et al., it is concluded that, IL-22 is a vital and important therapeutic molecule to enhance intestinal healing in patients with UC based on the observations in T cell receptor-alpha-deficient mice, wherein, IL-22 is over expressed, which ultimately reduced the disease score and colonic thickness in DSS-induced UC animal model (Sugimoto et al., 2008).

3. Multiparticulate formulation approaches for the colon targeting of the drugs using chitosan

Many drugs used in the treatment of IBD are associated with adverse effects, *viz.* Cushing's syndrome, glaucoma, osteoporosis, hepato- and nephrotoxicity, peptic ulcers, pruritus, diarrhea, pancreatitis, and malignancies. Optimal therapeutic efficacy and reduction of adverse effects of the drugs is the key for successful treatment of the disease and there is great probability of achieving this through multi-particulate drug delivery systems designed for the release of the drugs at the inflamed sites (Lautenschläger, Schmidt, Lehr, Fischer, & Stallmach, 2013; Rogler, 2010).

3.1. Chitosan containing pellets

Pellets have several advantages as a multiparticulate dosage form — i. Rapid transit through the upper GI tract in the presence of food if particle size is less than 2–3 mm, ii. Lesser chances of burst release, iii. No or minimal gastric irritation because of the distribution of an API in the stomach, and iv. Incompatible drugs can be encapsulated separately and mixed later, to name a few. Steckel H. et al. has reported development of chitosan pellets using extrusion-spheronization technique and explored possibilities of using chitosan as a major component of pellet formulation. Water and different concentrations of acetic acid was used as an extrusion liquid. Results suggested that 1:1 concentration of chitosan and microcrystalline cellulose (MCC) can be extruded successfully and with ease using water as granulating liquid; however, extrusion of 100% chitosan is possible only when higher acetic acid with normality of 0.2 N is used as granulating liquid (Steckel & Mindermann-Nogly, 2004). Recently, chitosan was reported to be used as a major component for preparation of pellets by extrusion spheronization technique for the colonic release of metronidazole, effect on the drug release was evaluated on uncoated and enteric coated pellets. It was concluded that, pellets containing chitosan showed extended release of the drug after enteric coating in gastric fluid as compared to the pellets devoid of chitosan. In contrast, there was no significant difference in the drug release when the pH of the dissolution medium was shifted to 6.8 (Ferrari et al., 2012). Chitosan was also employed as a pore former in the enteric coated core beads for the colonic delivery of 5-ASA in combination with ethylcellulose as a coating material for core beads. The criterion for taking the beads for further level of evaluation was set as not more than 10% drug released in simulated intestinal fluid (SIF) in 6 h. It was observed that as the level of chitosan goes up in the coating mixture, drug release was quicker. The reason cited was the hydrophilicity of chitosan, which swells in the presence of SIF while on the other hand ethylcellulose in sufficient quantity prevents wetting and swelling of chitosan and further resists entry of dissolution medium into dosage forms and slows down the drug release (Omwancha, Mallipeddi, Valle, & Neau, 2013).

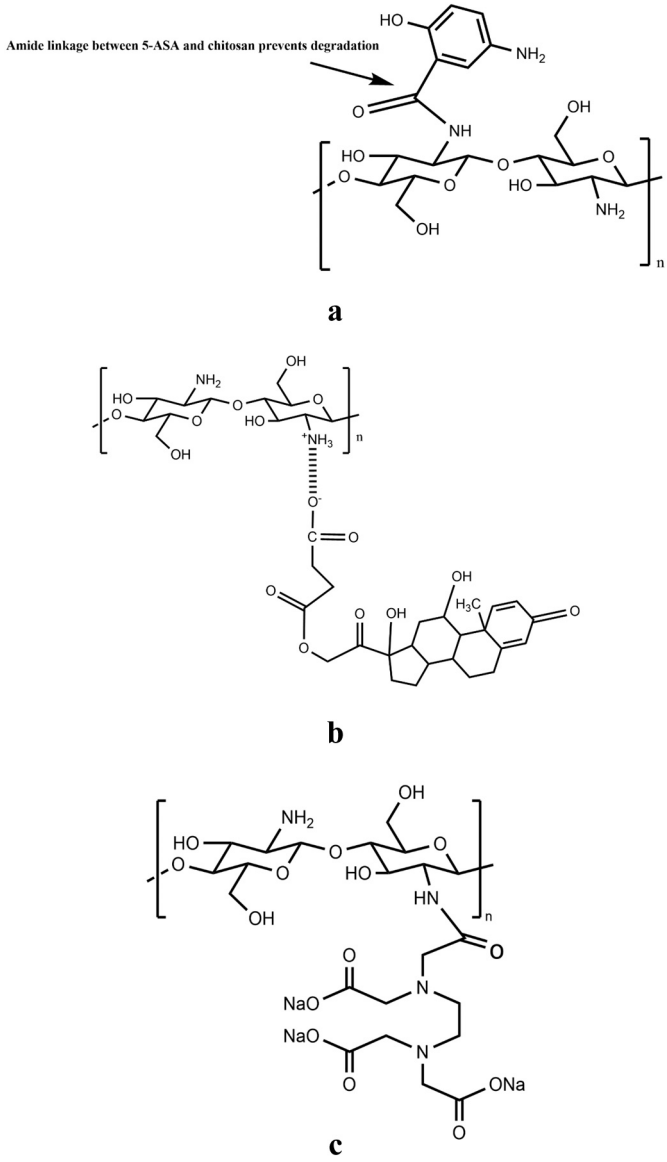

Fig. 5. a. Chemical structure representing the amide linkage between chitosan and 5-ASA. The amide linkage did not allow drug release because of amide bond stability hence the chitosan-5-ASA-azo linked compounds have been synthesized, b. Chitosan-Prednisolone succinate complex, and c. Chitosan-EDTA complex.

Controlled/sustained release of drugs after oral administration is important in prolonging action, reducing dosing frequency, and for the patient's convenience. Apart from sustained release applications, chitosan has been employed for delayed release of APIs in the pellet formulations. In one of the research reports, chitosan and pectin were used for the preparation of core pellets using extrusion-spheronization technique for controlling release of model drugs theophylline, dimenhydramine and ibuprofen; effect of porosity of pellets on the drug release was reported. Chitosan and pectin containing pellets have shown highest porosity against MCC containing pellets and this led to faster drug

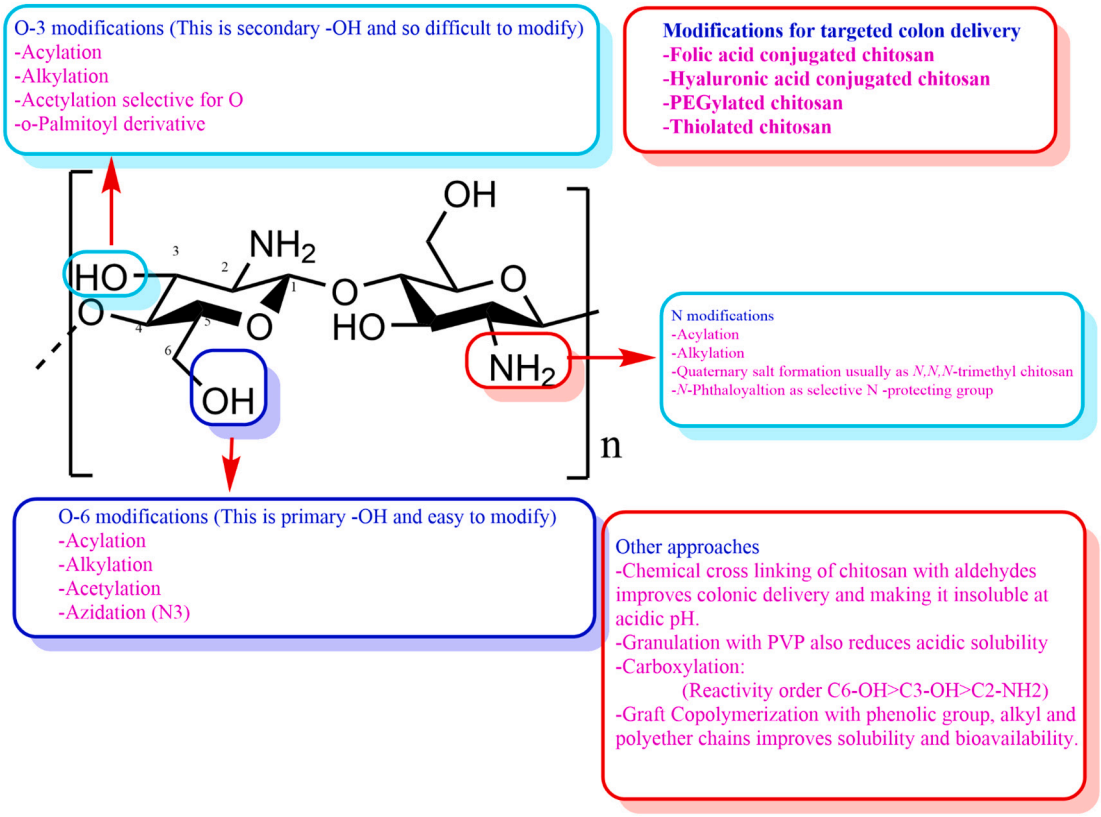

Fig. 6. Various approaches for modification in chitosan: Chitosan shows limited applications due to poor solubility in aqueous solutions. Hence, chemical modifications need to be done to chitosan so as to enhance solubility at physiological pH. Fig. 6 displays that chitosan can be chemically modified at O-3, O-6 and at N. The reactions at O-6 are easier to perform as this is primary hydroxyl group while the –OH at third position is secondary and difficult to modify. The figure represents the most common modifications that are performed to these three functional groups. Quaternization of NH_2 is important modification as it not only increases solubility but also improves absorption across biological membranes. Besides these common modifications, the figure also represents other approaches adopted for colon-targeted delivery.

release from the former as compared to the latter (Nejati et al., 2018). In another research work, surface coating by chitosan was evaluated for sustaining the drug release of an anti-diabetic drug Metformin hydrochloride, which was adsorbed onto the mesoporous silica nanoparticles. Chitosan paste was mixed with these mesoporous silica nanoparticles for the preparation of quasi-spherical pellets using molding technique and in the second stage five layers of chitosan coating was applied over these pellets to prevent release of the drug in the acidic pH. Surface coating led to prolongation of drug release in neutral pH, however, burst release was observed in pH 1.2, that resulted in 100% of the drug release at the end of 17 h of dissolution study as against merely 26% drug released in pH 7.0 in the same time (Patiño-Herrera et al., 2019). There are primarily four parameters, which govern the effect of chitosan on dissolution; these are degree of deacetylation, percentage content in a formulation, viscosity grade and solubility of an API. For an acidic drug piroxicam, chitosan was reported to have a solubility enhancing effect in acidic pH, this effect has shown to be increased exponentially with the grade of chitosan used. At the optimized ratio of chitosan and piroxicam, chitosan facilitated complete release in an extended manner over 8 h. (Partheniadis, Gkogkou, Kantiranis, & Nikolakakis, 2019). Chemical structure of Chitosan linked with 5-ASA, predisolone and Ethylenediaminetetracetic acid (EDTA) are shown in Fig. 5. Fig. 5b represents an electrostatic interaction between quaternary amine and carboxylate ion. This interaction takes place due to protonation of amine in aqueous acidic media that leads to reaction of chitosan bearing amine moiety with prednisolone succinate.

3.2. Chitosan microspheres

Amino group present on the chitosan undergoes protonation in the acidic environment due to which chitosan shows good solubility at acidic pH (Park, Saravanakumar, Kim, & Kwon, 2010). As mentioned in Table 1, there are marked changes observed in the pH gradient of the GIT in IBD patients and solubility of chitosan at acidic pH becomes an important property for colon specific release considering shift in the colonic pH towards acidic side. Table 2 lists the techniques used in the preparation of chitosan microspheres for encapsulation of various drug molecules having different aqueous solubility, targeted to the colon for alleviating symptoms of IBD.

3.3. Nanoparticulate drug delivery systems comprising of chitosan

Nanoparticles have been explored as a very effective tool for targeting the drugs to the inflamed sites. Owing to their small size they can accumulate in large quantities in the specific tissues. Drug targeting to the colon using nanocarrier approach can be achieved by different mechanisms either alone or in combination, these are time-, pH-, pressure- or gut microflora responsive. There were incidences wherein therapy has failed due to insufficient drug deposition or drugs have shown adverse effects because of the lack of release at the desired site, these untoward incidences can be prevented *via* nanoparticulate drug delivery. Over the years multiparticulate drug delivery systems especially micro- and nano- sized particles have proven ability of drug targeting to the specific sites in the GIT and this ability is further amplified because of the mucoadhesive characteristics of the polymers such as chitosan. Commonly used techniques for the preparation of chitosan

Table 2
Some examples of colon targeting of drugs achieved through chitosan comprising microspheres and summary of the results obtained.

Technique/s used for the preparation of microspheres	Polymers/material used alongside chitosan in the study	Drug/s encapsulated	Significant outcomes of the study	Reference
Spray-drying	–	5-ASA	■ Enhancement in the solubility of the drug, and improvement in intrinsic dissolution was observed. ■ Microspheres did not show cytotoxicity and reduced messenger RNA (mRNA) levels responsible for the release of IL-1β and IL-8.	(Aguzzi et al., 2011)
Spray-drying	–	5-ASA	■ N-succinyl-chitosan was explored for colon specific release because of its stability in acidic pH, biocompatibility, low toxicity and mucoadhesive property.	(Mura et al., 2012)
Spray-drying	–	Mesalazine	■ Chitosan microspheres were designed for the rectal administration of mesalazine to bypass the variable physiological environment in the GIT after oral administration. ■ In an *in-vitro* study chitosan microspheres showed certain degree of cytotoxicity at polymer concentration greater than 200 μg/ml. ■ Half of the dose of the drug given through microparticles as compared to marketed formulation Asamax® produced the same effects in alleviation of the disease.	(Palma et al., 2019)
Spray-drying followed by ionotropic gelation/ polyelectrolyte complexation	Sodium alginate	5-ASA	■ Prolong release of the drug was expected based on the physicochemical properties of the polymers *i.e.* mucoadhesiveness and pH sensitive solubility. ■ Physicochemical properties of the drug such as its p*Ka* (2.3 and 5.4) and log *P* (1.4) were also taken in to account to predict the release. ■ Microspheres prepared by using high viscosity alginate richer in mannuronic acid (M) content, exhibited faster release *i.e.* between 40 and 50% in the first 02 h of the study carried out in acidic conditions where drug has good solubility as well (8.65 mg/ml). ■ Slower release of the drug in phosphate buffer pH 6.8 was also attributed to its lesser solubility (3.94 mg/ml) in pH 6.8. ■ When alginate richer in guluronic acid (G) was used, slower release of the drug was observed as compared to alginate richer in M, in the first 08 h of the dissolution study. ■ This is due to, formation of high porosity, low shrinking ability and no swelling after drying (rigid) of gels having higher content of G. In contrast gels formed by the alginate having higher content of M are elastic, softer, shrink/swell more. ■ Microspheres exhibited dominant localization of 5-ASA in the colon and low systemic bioavailability in the bio-distribution studies.	(Mladenovska et al., 2007)
Ionic cross-linking and ionotropic gelation	β-Glucan and sodium alginate	Tylophorine malate (NK007)	■ A beta-glucan, glucan mannan particles (GMPs) were separated from the commercially available yeast cells (cell wall). ■ These GMPs were labeled with Rhodamine and then incubated with NK007 in chitosan solution for 2 h, to allow GMPs to swell and engulf NK007. Chitosan was cross-linked with mixture of solution of tripolyphosphate and sodium alginate. ■ Beta-glucans show specificity and selectivity for macrophages, when GMP containing microspheres were assayed for cellular uptake in RAW 264.7 cells, exhibited efficient internalization. ■ Intestinal uptake studies were carried out using mice model. These studies revealed efficient uptake of microspheres by epithelial cells into the intestinal mucosal layer. ■ Disease activity index (weight loss, stool consistency, and faecal bleeding) was measured in the DSS induced colitis model. GMP-NK007 microspheres and plain NK007 treatments were compared. It was observed that disease activity was controlled by both the treatments, but there was no significant difference between them. ■ The disease activity was controlled efficiently because of suppression of pro-inflammatory cytokine TNF-α.	(Chen, Wang, et al., 2015)
Crosslinking by TPP, polyelectrolyte complexation	Sodium alginate, pectin, Eudragit S 100	Ketoprofen, and ascorbic acid	■ Waxy materials and hydrophilic polymers were used for encapsulation. ■ Hydrophilic polymers showed good entrapment efficiency for both the APIs as compared to waxy materials. ■ Hydrophilic polymers despite cross-linking and combined use of two polymers (Chitosan-alginate) could not prevent	(Maestrelli, Zerrouk, Cirri, & Mura, 2015)

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Table 2 (*continued*)

Technique/s used for the preparation of microspheres	Polymers/material used alongside chitosan in the study	Drug/s encapsulated	Significant outcomes of the study	Reference
			release of ascorbic acid, more hydrophilic drug than ketoprofen, in gastric pH. ■ Microspheres made up of the hydrophilic polymers need to be enteric coated to prevent early drug release.	
Emulsification and gelation	Sodium alginate	5-ASA, and zinc	■ Microspheres exhibited pH dependent release of 5-ASA and Zn^{2+}, with higher percent release of the drugs at pH 7.4. ■ Increase in cross-linking by Zn^{2+} ions decrease release of 5-ASA. ■ Sharp decline in the clinical scores in colitis induced rat model after treatment with 5-ASA and Zn^{2+} containing microspheres was observed.	(Duan et al., 2017)
Emulsification (w/o) followed by crosslinking with glutaraldehyde	Eudragit S100	Aceclofenac	■ Eudragit S100 coating prevents early release of the drug at gastric pH. ■ Biodistribution studies revealed efficient targeting to the colonic site by the Eudragit S100 coated chitosan microspheres as compared to uncoated chitosan microspheres.	(Umadevi, Thiruganesh, Suresh, & Reddy, 2010)
Emulsification followed by cross-linking	Eudragit S100	5-ASA, camylofine dihydrochloride	■ There was no significant difference observed in the drug release when evaluated in gastric fluid and phosphate buffer saline pH 7.4. ■ Significant increase in the drug release was reported when 3% rat caecal contents were used in the dissolution medium. ■ Enhanced release of both the drugs was observed after colonic enzymes were induced in the rat by oral administration of chitosan. ■ Microspheres released the drugs after a lag time of 9 h; hence possesses drug targeting potential to the colon.	(Dubey, Dubey, Omrey, Vyas, & Jain, 2010)
Emulsion cross-linking	Eudragit S-100	Curcumin	■ pH sensitive microspheres of curcumin were prepared for colon specific drug release. ■ Microsphere showed high entrapment efficiency over the range of 74–83% across all formulation batches. ■ Chitosan microspheres displayed burst release in the first four hours of dissolution study, to minimize this quick release, was later coated with Eudragit S100. ■ Curcumin containing microspheres exhibited better control over disease activity in acetic acid induced colitis model.	(Sareen, Jain, Rajkumari, & Dhar, 2016)
Emulsification internal gelation	Sodium alginate	Icariin	■ A flavonoid, Icariin, which has poor solubility and low bioavailability, was encapsulated successfully in the microspheres. ■ Fluorescence labeled microspheres indicated high retention in the colon for more than 12 h. ■ Microspheres effectively reduced colon mucosa damage index and also reduced production as well as gene expression of inflammatory mediators and cytokines in 2,4,6-trinitrobenzenesulfonic acid (TNBS)/ethanol induced colonic inflammation.	(Wang, Wang, Zhou, Gao, & Cui, 2016)
Water-in-oil (w/o) emulsification and cross-linking with glutaraldehyde	Acrylamide grafted chitosan polymer	5-ASA	■ Acrylamide grafted chitosan (CHI-g-AAm) polymer was synthesized for colon specific drug delivery. ■ Drug release was significantly higher in the simulated colonic fluid containing caecal and colonic content against the simulated stomach and small intestinal fluid. ■ Microspheres were evaluated for their healing capacity against 2,4,6-trinitrobenzene sulfonic acid sodium salt induced colitis in rats. ■ Colonic inflammation was assessed by measuring Myeloperoxidase activity, colon/body weight ratio and damage score. ■ CHI-g-AAm microspheres showed better activity against all the mentioned parameters as compared to the drug solution administered orally.	(Jain et al., 2008)
Emulsification cross-linking and emulsion solvent evaporation	Eudragit	Sinomenine	■ Newly developed microspheres were evaluated in DSS induced mice model. ■ The disease activity index was measured on the basis of combined scores of weight loss, stool consistency, and bleeding, which was found to be insignificantly lower in sinomenine microspheres group than in plain sinomenine group. ■ Immunohistochemistry and real-time polymerase chain reaction (PCR) studies for the expression of TLR4, Myeloid differentiation primary response 88 (MyD88) and NF-κBp65 revealed lowering in the expression in the animal groups treated with sinomenine-chitosan and sinomenine enteric microspheres treated groups in comparison to plain sinomenine and salicylazosulfapyridine treated groups.	(Xiong et al., 2017)

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Table 2 (*continued*)

Technique/s used for the preparation of microspheres	Polymers/material used alongside chitosan in the study	Drug/s encapsulated	Significant outcomes of the study	Reference
Emulsification cross-linking and emulsion-solvent evaporation	Eudragit S 100 and halloysite nanotubes	Paeoniflorin	■ The halloysite nanotubes (HNT) contain hydroxyl and siloxyl groups on its surface which assists in adsorption of various chemicals and drugs. ■ Chitosan, being cationic, spontaneously binds to the negatively charged surface of HNT. ■ Paeoniflorin containing HNT/Chitosan microspheres were prepared by W/O emulsification technique and chitosan was cross-linked using glutaraldehyde. ■ Secondary coating of Eudragit S100 was applied over the microspheres using emulsion solvent evaporation technique. ■ Presence of chitosan facilitated permeation of microspheres in the colonic tissue, evaluated through everted gut sac technique.	(H. Li et al., 2021)
Emulsification and solvent evaporation	Eudragit L 100 and Eudragit S 100	Prednisolone, and prednisolone 21-hemisuccinate sodium salt	■ Prednisolone 21-hemisuccinate was conjugated with chitosan and chitosan microspheres of the conjugate were prepared which were then coated with the enteric polymer. ■ No significant difference in the drug release was observed at pH 1.2 between plain chitosan and enteric polymer coated chitosan microspheres. This is due to slow hydrolysis of the ester bond present in the conjugate. ■ Drug release was quicker in plain chitosan microspheres than enteric polymer coated microspheres at pH 6.8 due to quick hydration of the former.	(Oosegi, Onishi, & Machida, 2008)
Emulsification and solvent evaporation	Eudragit L 100	Prednisolone 21-hemisuccinate sodium salt	■ In continuation to the previous work, Eudragit L 100 coated microspheres were prepared and evaluated in TNBS induced colitis model. ■ On the basis of measurement of different inflammation indices such as myeloperoxidase (MPO) activity and ratio of proximal colon weight to body weight and distal colon weight to body weight, it was concluded that enteric coated microspheres has better efficacy and lesser toxicity than plain chitosan microspheres.	(Onishi, Oosegi, & Machida, 2008)
Emulsion solvent evaporation technique based on multiple emulsion (w/o/w)	Cellulose acetate butyrate (CAB)	5-ASA	■ Here, chitosan was selected as a drug carrier material based on its ability to undergo biodegradation by the enzyme lysozyme present abundantly in the colon, secreted by colonic microflora. ■ Core chitosan microspheres were coated with Cellulose acetate butyrate (CAB) for preventing the drug release in the acidic environment. ■ Chitosan molecular weight and core/coat ratio of the polymers has significant effect on the % bioadhesion of the microspheres, which was performed using averted sac technique. ■ Rat caecal content has positive effect on the release of the drug from microspheres; this is due to biodegradation of chitosan, and reduction of the pH by the products of bacterial fermentation.	(Varshosaz, Jaffarian Dehkordi, & Golafshan, 2006)
Emulsification solvent evaporation (o/o)	Eudragit S 100 and Ethylcellulose	5-ASA	■ Blend of ethylcellulose and Eudragit S 100 was used in a few formulation batches for the preparation of microspheres. ■ Production yield obtained for all the batches was very high ranging from 84 to 99%. ■ Reduction in concentration of Eudragit S 100 in the internal phase of an emulsion led to significant decrease in the particle size distribution due to increase in shearing action as a result of decreased viscosity of the internal phase. ■ A combination of time dependent approach and pH dependent approach using ethylcellulose and eudragit S 100 respectively was explored to prolong the drug release after reaching the colon. ■ Optimized batch of formulation released minimum amount of 5-ASA in first 4 h of dissolution studies, and drug release was prolonged over 12 h after reaching the colon.	(El-Bary, Aboelwafa, & Al Sharabi, 2012)
Emulsion polymerization	Wheat germ agglutinin	Reduced brominated derivative of noscapine	■ Chitosan microspheres were prepared by emulsion polymerization method and later coated with wheat germ agglutinin for enhancement of bioadhesive properties. ■ Microspheres exhibited affinity towards colonic mucin secreting cells in simulated colonic fluid of ~pH 7.2. ■ Microspheres showed pH sensitive release of the drug in simulated colonic fluid with colonic milieu (pH ~ 4.7).	(Kaur et al., 2015)
Single-step electrospraying	Sodium alginate	IL-1 Ra (Recombinant IL-1 receptor antagonist)	■ Microcapsules were prepared by single-step electrospraying technique and sodium alginate coating was hardened with Ca^{2+} ions in the presence of chitosan.	(Cao et al., 2019)

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Table 2 (*continued*)

Technique/s used for the preparation of microspheres	Polymers/material used alongside chitosan in the study	Drug/s encapsulated	Significant outcomes of the study	Reference
			■ Microspheres showed pH dependent drug release, with slow and smaller amount was released in simulated gastric fluid. In simulated intestinal fluid drug release shot up to 86% within 2 h of the study. ■ Treated mice with microspheres showed decrease in the disease activity estimated on the basis of disease activity index which was evaluated in dextran sulphate (DSS) sodium induced colitis model. ■ IL-1 Ra containing microspheres exhibited improvement in the damaged colonic tissue evaluated by histological studies. ■ Serum concentration of different cytokines such as TNF-α, and IL-1β was evaluated in animal model, post treatment concentration of the cytokines was found to decrease significantly.	

nanoparticles include ionic gelation, spray-drying, emulsion followed by cross-linking and complex coacervation (Saboktakin, Tabatabaie, Maharramov, & Ramazanov, 2011).

Scalability of nanoparticles has always been challenging for formulation scientists across the world and manufacture must be supplemented with narrow particle size distribution. To address the issue, Huanbutta et al. had developed chitosan nanoparticles coated with poly (methyl acrylates) using spinning disc processing technique. High entrapment efficiency of 88% was reported with over 90% drug release in the simulated colonic fluid within 8 h. With this novel approach, researchers achieved selective targeting to the colon with minimum potential of drug release in the upper GIT (Huanbutta et al., 2013).

3.3.1. *Colonic delivery of small molecules via chitosan-based nanoparticles*

Mucus is indigenous to the intestine majorly made-up of mucin, a glycosylated glycoproteins possessing negative charge. Specialized cells known as Goblet cells secrete the mucus. Alteration in the number of goblet cells and reduction in the thickness of the mucus is observed in the IBD (Brown, Whitehead, & Mitragotri, 2020; Michielan and D'Incà, 2015). Mucus varies in thickness and level of adherence, it has thickness of 13–167 μm of firmly attached layer and 97–823 μm of loosely bound layer on the surface (Hunter, Elsom, Wibroe, & Moghimi, 2012). Chitosan is a cationic polymer, nano-carriers made-up of it interact with the mucus by electrostatic interaction and exhibit prolonged residence time at the desired site, which is advantageous considering rapid bowel movements in IBD, over and above this property also enables higher drug permeation across the epithelium (Grenha, 2012; Hua et al., 2015a). Production of mucus is reported to be high in active UC, and as mentioned, chitosan containing nanoparticles show adherence to the mucus, owing to their small size nanoparticles are taken up well by the macrophages in an inflamed area. Mongia et al. had developed mucoadhesive chitosan nanoparticles of curcumin for the treatment of UC. Nanoparticles were prepared by ionic gelation technique using tripolyphosphate as a cross-linker. The only limitation of chitosan for colon targeting when administered *via* oral route its protonation in the acidic environment of the stomach that leads to its solubilization, here, this problem is addressed by coating of nanoparticles by Eudragit FS 30 D. Because of the coating nanoparticles showed good accumulation in the colonic area as revealed in the biodistribution studies carried out using gamma scintigraphy (Raj, Raj, Kaul, Mishra, & Ram, 2018). On the similar lines, chondroitin sulphate functionalized nanoparticles of curcumin were developed for targeting colonic macrophages, for their protection in harsh conditions of upper GIT, nanoparticles were encapsulated in chitosan-alginate hydrogel (Zhang et al., 2019). Natural polymers for the site-specific drug delivery to the colon have grabbed more attention over the past few years due to low toxicity as compared to the synthetic polymers. In conditions like colon cancer and Crohn's disease, increased secretion of the mucus suits well for mucoadhesion and this ultimately leads to increased flux of the drugs across colonic mucosa. In an attempt to increase propensity of mucoadhesion, Sabra et al. have reported modified pectinate-chitosan nanoparticles prepared by ionic gelation technique. Mucin, from the porcine stomach at four different concentrations 10, 50, 100 and 150 μg/ml was used to evaluate the mucoadhesion propensity. Change in the zeta potential of the nanoparticles was evaluated after incubating them in simulated pH media for stomach and colon *i.e.* pH 1.2 and 7.0, respectively. Authors reported that zeta potential reduces to +18.2 mV from +35.7 mV in acidic conditions and to +1.22 mV in pH 7.0. From the above readings, nanoparticles were inferred to be stable in acidic conditions; however, at neutral pH drastic reduction in the zeta potential is due to Van der Waals inter-particle attraction and this aggregation affects mode of cellular uptake and biological response. Further, drop in zeta potential can be attributed to electrostatic interaction between positive charge of chitosan and negative charge of sialic acid moieties of the mucin. Therefore, significant drop in the zeta potential indicates extent of mucoadhesion. It is concluded that pectinate-chitosan nanoparticles are more mucoadhesive at neutral pH than acidic (Sabra, Roberts, & Billa, 2019). In another research work, chitosan-dextran sulfate nanoparticles were evaluated for the colonic delivery of a model drug 5-ASA. Sustained drug release over the period of 10 h. was reported, and there was no significant effect of chitosan-dextran sulfate observed to be on the drug release (Saboktakin, Tabatabaie, et al., 2010). On the similar line, same research group has reported successful delivery of a model drug 5-ASA to the colon *via* carboxymethyl starch-chitosan nanoparticles (Saboktakin, Maharramov, & Ramazanov, 2010).

Chemical modification of chitosan is reported in various studies for improvement of the physicochemical properties of the natural polymer, especially to improve its solubility over a wide pH range. In one such study, for the colon targeting of low molecular weight heparin (LMWH) which has strong therapeutic activity in UC because of its anti-inflammatory, anticoagulant and mucosal healing effects, authors developed trimethyl chitosan (TMC) and sodium alginate coated-TMC (SA-TMC) nanoparticles containing LMWH. There are several issues associated with oral administration of heparin- low bioavailability due to poor absorption in the GIT, high anionic charge density, first pass metabolism, and enzymatic degradation. Therefore, colon targeted nanoparticulate delivery offers an advantage in terms of passive targeting to the inflamed colon owing to enhanced permeability and retention effect. As an outcome, *in-vitro* mucosal permeation study revealed significantly enhanced passage of the drug across rat intestine by 3.45 fold through TMC nanoparticles and 2.67 fold through SA-TMC nanoparticles when compared against free LMWH. This enhancement is attributed to reversible opening of tight junctions of epithelial cells mediated by chitosan-based nanoparticles (Wang et al., 2017; Wang &

Kong, 2017; Yeh et al., 2011). Furthermore, anticoagulant effect of LMWH was determined *in-vitro* in human plasma; nanoparticles exhibited significant extension of the prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) as compared to normal saline. In continuation, for determination of effect of nanoparticles on oral absorption of LMWH in rats, *in-vivo* studies were performed by activated partial thromboplastin time (APTT) assay. In the study, TMC nanoparticles exhibited highest anticoagulant activity, maximum APTT significantly prolonged to 2 h, which is 1.60 fold rise as compared to free LMWH, this observation highlights strong anti-coagulant activity and better oral absorption. On the other hand, SA-TMC nanoparticles showed 1.31 fold increase in APTT value. This significant rise in ATPP through TMC nanoparticles was due to the cationic charge that facilitated penetration of LMWH *via* paracellular transport thus improving oral absorption and anti-coagulant activity. In the pharmacodynamics, ameliorative effects of nanoparticles was evaluated in TNBS induced colitis mice model. Here, nanoparticles treatment group exhibited significantly positive effect on some key indicators of IBD as in better control over weight loss, reduced disease activity score, restoration of the colon length and finally, decline in MPO activity as compared to free LMWH (Mittal et al., 2018; Yan Yan et al., 2020).

Chitosan and its derivatives are widely used for coating liposomes in achieving site specific gastrointestinal delivery because of their mucoadhesive/bio-adhesive property (Fatouh, Elshafeey, & Abdelbary, 2020; Huang, Wang, Chu, & Xia, 2020; Tai et al., 2020), however, chitosan alone cannot protect the lipid vesicles in the acidic environment of the stomach. In a novel approach, Castingia et al. reported colonic delivery of quercetin, a potent anti-inflammatory and antioxidant drug, achieved through its encapsulation into phospholipid vesicles (nanoparticles) coated with chitosan/nutriose. Phospholipid vesicles are prone to acidic pH and enzymatic degradation that can be protected by employing coating of suitable polymers. Herein, chitosan and nutriose was used for coating of nanovesicles. These nanosystems exhibited better control of the disease activity (weight loss, and rectal bleeding) over uncoated ones as revealed through a pre-clinical study using rat model in which IBD was induced with the help of TNBS (Castangia et al., 2015).

In a recent study, researchers have proposed novel chitosan coated c-SLN (Solid-lipid nanoparticles) delivery system. This research group have designed an effective hybrid system for chemoprevention, which is the combination of SLNs and chitosan-based drug delivery systems. c-SLNs have wide range of pharmaceutical applications and employed as a promising vehicles for oral drug delivery systems because of their characteristics such as permeation enhancer, bioadhesion and biodegradation. In addition, cationic nature of chitosan leads to prolonged residence time at the negatively charged epithelia in the small intestine that shows significantly enhanced drug concentration at the site of absorption. In addition, chitosan can facilitate reversible opening of the tight junctions between neighboring epithelial cells, promoting drug molecule's paracellular transport, thereby enhancing bioavailability of the encapsulated drugs. In conclusion, chitosan as a drug delivery vehicle, and SLNs combine the advantages of both the carrier materials (Fonte et al., 2012; Thakkar, Chenreddy, Wang, & Prabhu, 2015).

3.3.2. *Chitosan nanoparticles in the colonic delivery of macromolecules*

Therapy of IBD with macromolecules has a unique advantage in addressing the underlying cause of the inflammation as compared to conventional treatments using small molecules. Macromolecule therapy options include immunosuppressants such as cyclosporine-A, anti-TNF-α agents *e.g.* infliximab, adalimumab, certolizumab pegol, natalizumab, interleukin-12/23 antibodies, interleukin-6-receptor antibodies, vaso-active intestinal peptide, bioactive natural peptides *e.g.* Lys-Pro-Val (KPV), and RNA based therapeutics. Presence of gastric acid and proteolytic enzymes in the GIT that would degrade macromolecules administered orally without any protection, in addition to that colonic microbiota-mediated metabolism, mucus layer, intestinal epithelium and basement membrane limits oral administration of macromolecules. Aforementioned advantage of macromolecules suffers a major blow due to no alternative route other than injectable is available for their systemic administration. Altered structural intactness of the apical side of the gastrointestinal mucosa in IBD provides an opportunity for oral delivery and localization of macromolecules (Stallmach, Hagel, & Bruns, 2010; Zhang, Thanou, & Vllasaliu, 2020). Selective drug targeting to the desired tissue/site is a key in the treatment of the IBD. Conventional drug delivery systems are unable to release the required amount of the drug in the colon; apart from that, drug absorbed in the upper part of the GIT produces adverse reactions and side effects considering long-term medications in the IBD patients. Therefore, a new drug delivery system that can release the drugs in the colon is the need of the hour. Polysaccharide polymers are ideal for colon-targeting of the payloads because of their degradation by the enzymes produced by the microflora and structural modifications of these polymers is also an formidable alternative (Chen et al., 2020; Deng et al., 2019; X. Li, Lu, Yang, Yu, & Rao, 2020). In an attempt to target anti-inflammatory tripeptide KPV to the colonic region Laroui et al. have developed biodegradable nanoparticles which were further encapsulated into polysaccharide hydrogel made-up of chitosan and alginate. The polysaccharides specifically selected because of their ability to protect the payloads from the harsh acidic environment of the stomach and ability to degrade by the colonic microflora, which makes site-specific delivery of macromolecules possible. *In-vitro* interaction between the nanoparticles and Caco 2-BBE (brush border enterocytes) showed drug release in the vicinity and inside the cell membrane (intracellular space). Similarly, LPS induced inflammatory response in Caco 2-BBE was controlled by downregulation of inflammatory cytokines in the dose dependent manner by the drug-loaded nanoparticles. *In-vivo* studies in DDS-induced colitis mice model revealed improved disease activity index, and reduced intestinal inflammation, which was further confirmed by determination of pro-inflammatory cytokines (downregulated) in the colonic tissue. Dose of KPV reduced by 12,000 times post encapsulation into nanoparticles, probably due to endocytosis of the nanoparticles by epithelial cells and drug release in the extra- and intra-cellular space. KPV nanoparticles encapsulated in hydrogel matrix prevented early drug release in the upper GIT hence facilitated drug deposition at the inflamed site. Further, high level mucus secretion is reported during intestinal inflammation, here, chitosan played its role in enhancing anti-inflammatory response of the drug by interacting with the mucus owing its cationic charge hence maintaining the drug molecules at the inflammation site for longer duration (Laroui et al., 2010). In another research work, Rivera et al. have reported sequentially assembled hollow nanocapsules made-up of chitosan-alginate developed by using layer-by-layer deposition technique by exploring opposite charges on the polymers. The technique is inexpensive, adaptable and easy to perform; in the study glycomacropeptide and 5-ASA was used as model drugs. This study focuses on finding release mechanisms of both the drugs from the nanoscale particles; concludes that release of the drugs followed i. Fickian diffusion, and ii. Polymeric relaxation, which is due to hydrophilic nature of the polymers. At pH 7.0, the anionic alginate from the complex may have been displaced by hydroxyl ions and chitosan losing its positive charge facilitating quick release of encapsulated drugs (Rivera, Pinheiro, Bourbon, Cerqueira, & Vicente, 2015). Chitosan offers several advantages for the delivery of small interfering RNA (siRNA), such as bioadhesion, biodegradability, and strong affinity for nucleic acid. The modification of primary amino groups of chitosan with glycidyltrimethylammonium chloride gives quaternary chitosan. This quaternization has shown enhanced nucleic acid binding capacity and improves cellular uptake by facilitating electrostatic affinity between chitosan and cell membrane (Xiao et al., 2017). However, its solubility at acidic pH and insolubility at neutral and alkaline pH limits its utility for the colon specific drug delivery. Trimethylation of chitosan enhances its solubility over a wide pH range and incorporation of thiol group further improves its bioadhesion through covalent bonding with mucin

glycoproteins thereby facilitates cellular uptake and gene transfection efficiency by solubilizing at extra- and intra-cellular pH. TNF-α, a pro-inflammatory cytokine plays central role in progression of IBD. Monoclonal antibody treatment is worthy, but also has high cost and side effects. Knockdown of functional proteins at mRNA level mediated by siRNA provides another approach for the treatment of various inflammatory diseases due to its high specificity and efficacy. For targeting mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4) a siRNA, which is known as key upstream mediator of TNF-α action, to the macrophages at the inflamed colon Zhang et al. have reported galactosylated TMC nanoparticles for oral delivery. Owing to the high affinity between galactose residue and macrophage galactose-type lectin (MGL) receptors expressed on the surface of macrophages, nanoparticles exhibited good binding affinity towards them when evaluated in *in-vitro* cell culture of Raw 264.7 cells, similarly, significant knockdown of TNF-α secretion was observed in LPS-stimulated Raw 264.7 cells. These nanoparticles significantly suppress TNF-α production in DSS-induced colitis mice tissue, improved disease activity in the animals, and inhibited MPO activity (Zhang, Tang, & Yin, 2013). Further, in a similar approach Huang et al. reported TNF-α siRNA containing nanoparticles encapsulated within the poly lactic-*co*-glycolic acid (PLGA) matrix by employing a coating of galactosylated chitosan to target at MGL (Huang, Guo, & Gui, 2018). There are two best characterized phenotypes of macrophages, M1 and M2 believed to have a vital role in the inflammation. M1 macrophages are responsible for secretion of pro-inflammatory cytokines and subsequently worsening of the situation, whereas M2 macrophages are involved in tissue repair. M1 macrophages are dominant in the early stage of inflammation later on they are replaced by M2 phenotype. MiR146b is up-regulated in human monocytes and acts as an anti-inflammatory agent by inhibiting TLR4 signaling pathway, it also targets interferon regulatory factor (IRF5) and thereby inhibit activation of M1 macrophages. Considering this, mannose-modified TMC nanoparticles (MTC), which are taken up by macrophages *via* mannose receptor-mediated endocytosis, are conjugated with miR-146b mimic. Herein, Deng et al. explored molecularly targeted immunotherapeutic strategy using MTC for inhibition of M1 macrophage activation and subsequent pro-inflammatory cytokine release; in an attempt to promote mucosal healing and suppress the development of colitis-associated carcinoma. As compared to negative control MTC, MTC- miR-146b mimic nanoparticles significantly inhibited inflammation and promoted epithelial regeneration in DSS-induced colitis mice model (Deng et al., 2019; He et al., 2016). Nearly 25% of the patients treated with monoclonal antibody, infliximab, suffered from at least one of the serious adverse effects, namely pneumonia, cancer or acute inflammation, may probably be due to lack of drug targeting and hence taking over dose of the drug. As mentioned, TNFα plays crucial role in the progression of inflammation, Laroui et al. had reported TNFα siRNA loaded biodegradable nanoparticles made up of poly (lactic acid) poly (ethylene glycol) block copolymer for macrophage targeting by covalently attaching Fab' portion of F4/80 antibody on to the surface. These nanoparticles were further coated with chitosan-alginate as this hydrogel collapses at pH 5 or 6 and ensures colon specific drug release. Phagocytosis of Fab′ portion bearing nanoparticles by macrophages took place very quickly when evaluated *in-vitro* on RAW 264.7 cells, similarly these nanoparticles reduced TNF-α expression in inflamed macrophages. Hydrogel-encapsulated Fab'-bearing TNF-α siRNA-loaded nanoparticles improved disease activity index and attenuated inflammation in DSS induced colitis mice model. TNF-α is a major upstream regulator of the NF-κB pathway; authors evaluated concentration of IKβα protein, an inhibitor of NF-κB, in the colon of DSS induced colitis mice model. Concentration of IKβα protein found to be higher in mice administered with Fab'-bearing siRNA-loaded nanoparticles as compared to Fab'-bearing scrambled siRNA-loaded nanoparticles, indicating that chitosan-alginate coating enabled cell specific accumulation of the siRNA thereby allowing disease attenuation by targeting an activity at the molecular level (Laroui et al., 2014). In another study, Wu et al. developed PLGA nanoparticles containing cyclosporine-A functionalized with KPV, a tripeptide targeted at oligopeptide transporter receptors (PepT1) that are overexpressed only in inflammatory condition on colonic epithelial cells and macrophages. Nanocarriers further coated with montmorillonite/chitosan for preventing early drug release at the acidic pH of the stomach. Montmorillonite chosen as a coating material because of its ability to interact with mucin by transforming into viscous gel; similarly, chitosan is well established as a coating material due its mucoadhesion ability and anti-inflammatory effect. It was hypothesized that these coated nanoparticles would increase retention time *via* time-, pressure-, pH-, or bacteria responsive mechanism and hence would target the colonic site. Fluorescent dye tagged nanocarriers exhibited 23-fold higher concentration in the inflamed colon than that in the healthy colonic tissue. Chitosan/montmorillonite coating contributed in the gathering of the nanocarriers at the inflamed sites by interacting with glycoprotein and mucin that made oral site-specific drug delivery possible thereby avoiding systemic distribution of the drug. In addition to that, PLGA-KPV could bind to macrophages and colonic epithelial cells and transported into the inflammatory colon cells *via* PepT1 as proven through confocal microscopic studies. In acute DSS induced colitis mice model, mRNA levels of TNF-α and IL-1β evaluated using RT-PCR, found to be significantly lowered in the nanoparticle treatment group when compared with marketed preparations of cyclosporine-A (Sandimmune). Interestingly, not just cyclosporine-A loaded nanoparticles exhibited anti-inflammatory response but, blank nanoparticles also exhibited the same response, probably due to synergistic action of KPV, MMT and chitosan. Of note, chitosan is reported to have anti-inflammatory response comparable to prednisolone (Wu et al., 2019). Higher than usual intestinal expression of NF-κB considered as important factor for progression of IBD, which is associated with altered intestinal barrier function and activation of pro-inflammatory signaling. On the other hand complete ablation of intestinal p65 (a subunit of NF-κB) expression in mice led to deregulation of response to injury and inflammation. Therefore, drug molecules balancing both sides *i.e.* inhibition of NF-κB but not to the extent of its abolishment would be an optimal therapeutic option. Prohibitin, a protein responsible for various cellular processes such as protein folding, proliferation control, suppression of oncogenesis, mitochondrial functions, and regulation of transcription processes, its expression is downregulated in UC and CD. Prohibitin has antioxidant and anti-inflammatory activity, its sustained expression in intestinal epithelial cells decreases TNF-α-stimulated NF-κB activation *in-vivo*. In the research work reported by Theiss et.al, prohibitin was deliver to the colon through adenovirus-directed administration *via* enema and orally through PLGA nanoparticles coated with chitosan-alginate hydrogels. Both the methods of administration of prohibitin proved beneficial *in-vivo*. Increased level of prohibitin was observed in the colonic tissue leading to reduced severity of DSS-induced colitis in mice model revealed through improved disease activity index, reduced MPO activity, reduced pro-inflammatory cytokine expression, improved histological score and reduced oxidative stress. pH- and time-dependent collapse of alginate-chitosan hydrogel enabled oral site-specific drug delivery of prohibitin thereby avoided its non-specific uptake that would have happened had it been administered *via* intravenous route (Theiss et al., 2011).

CD98, a type II transmembrane glycoprotein transporter over expresses on the surface of colonic epithelial cells and on intestinal macrophages in inflammation, it plays a vital role in the activation of the latter, thereby in the progression of IBD. The cytoplasmic domain of CD98 can interact with β_1- integrin and regulates cell homeostasis, epithelial adhesion and immune responses. Targeted drug delivery that can block CD98 could be a potential therapeutic target to ameliorate the disease progression. An effective tool to curb inflammation in this case is RNA interference (RNAi) *via* siRNA, which brings about post-transcriptional gene silencing (genes related to the disease) and inhibits CD98 expression on the macrophages. To exhibit its potential effects, siRNA has to enter cytoplasm by crossing cell barrier where it

can cause sequence-specific deterioration of mRNA. For entering in to the cytoplasm, a siRNA has to avoid degradation mediated *via* acidic endosomes/lysosomes. This degradation can be avoided by proton bearing buffering constituents, such as imidazole group containing compounds, polyethylenimine and chloroquine by disrupting endosomal/lysosomal membranes. Mucus is another barrier that resist localized drug delivery to the colonic mucosal surface when taken by oral route. Chitosan is biocompatible, biodegradable and positively charged that can form complex with siRNA by polyelectrolyte complexation which can be transformed into nanoparticles, apart from this chitosan has high transfection efficiency (Xiao et al., 2014; Yan, Yutao, Vasudevan, Nguyen, & Merlin, 2008). Considering this, Xiao et al. have reported uronic acid modified chitosan nanoparticles containing siCD98 as payload using complex coacervation technique. Single-chain CD98 antibody was conjugated to the surface of the nanoparticles using polyethylene glycol to enhance interaction between functionalized nanoparticles and CD98 protein. Nanoparticles were evaluated for uptake by colon-26 and RAW 264.7 cell lines, found to be taken-up very rapidly through active targeting; similarly, significant knockdown of CD98 expression was reported in the same cell lines as compared to scrambled siRNA loaded nanoparticles. A T-cell transfer and DSS-induced colitis mice model was explored for *in-vivo* studies. Antibody functionalized nanoparticles embedded in chitosan-alginate hydrogel after oral administration reduced weigh loss in the treatment group of mice in both type of colitis mice model. There was also significant reduction in MPO activity, and of CD98 expression in the treatment group (Xiao, Ma, Viennois, & Merlin, 2016). Further, in an attempt to treat UC through a combination therapy, Xiao et al. reported PLGA nanoparticles containing siTNF-α (a siRNA) and IL-22, a pro-healing cytokine, embedded in chitosan-alginate hydrogel for the colon targeting. Nanoparticles encapsulated in the hydrogel exhibited very strong inhibition of pro-inflammatory factors and promoted mucosal healing *in-vivo* in DSS induced colitis mice model (Xiao et al., 2018).

Eggshell membrane, formed as a one of the byproducts during egg processing, has demonstrated inhibitory effect on production of TNF-α and other pro-inflammatory cytokines such as IL-1β, MCP-1 (monocyte chemoattractant protein), MIP-1α and β (Macrophage inflammatory protein), RANTES (regulated upon activation, normal T cell expressed and presumably secreted), and VEGF (vascular endothelial growth factor). For its targeted delivery to the colon, Chen et al. reported novel chitosan-fucoidan nanoparticles. Fucoidan, an anionic polysaccharide extracted from brown seaweeds, used for the cross-linking of chitosan. Nanoparticles exhibited delayed release in the dissolution conditions mimicking GI pH, and strong antioxidant, immunomodulatory activity evaluated *in-vitro* (Lee & Huang, 2019).

US- food and drug administration (US-FDA) has approved several biosimilars that are used in the treatment of IBD; they are listed in Table 1 in the supplementary material. There are some research articles available in the literature on the colon specific drug delivery *via* oral administration of Infliximab using various available polymers (Foong, Patel, Forbes, & Day, 2010; Gareb et al., 2021; Maurer et al., 2016; Pabari et al., 2019). However, as they are out of the purview of this review hence not discussed here.

4. Chitosan-drug conjugates for the colon targeting

Of the several approaches employed for the colon targeting, drug-polymer conjugation *via* covalent linking for the production of the prodrugs is explored in the several marketed technologies. This approach is advantageous considering that it clubs physicochemical properties of drugs, polymers and physiological conditions of the GIT (Shahdadi Sardo et al., 2019).

To explore benefits of biodegradability, biocompatibility and GRAS status associated with chitosan, Nalinbenjapun et al. reported chitosan-5-ASA azoconjugate for colon specific drug release. Previously, Zou and co-workers had reported the same conjugate, but the conjugation was carried out through an amide bond between 5-ASA and chitosan. This conjugate failed to release the drug in simulated gastrointestinal fluid containing rat caecal or colonic content. The failure was attributed to a very stable amide bond, which did not break during *in-vitro* dissolution studies. However, the newly developed azo-conjugate was stable in *in-vitro* stability studies carried out using simulated gastric, intestinal and colonic fluids indicating it would avoid premature drug release in the upper GIT. *In-vitro* release studies demonstrated that there was no drug release from the conjugate in simulated gastric conditions over 24 h and in simulated intestinal conditions over 6 h, 15% of drug released over 6–24 h. In simulated colonic fluid, 10% of drug released over 6 h and maximum drug release was 25% over 24 h. Insufficient drug release in comparison to sulfasalazine (around 70%) is due to protection of the azo bond from enzymatic attack by steric hindrance brought about by the large molecule (Nalinbenjapun & Ovatlarnporn, 2020; Zou et al., 2005).

Amphiphilic polymers have attracted the attention of drug delivery scientists over the last few years. Especially amphiphile made-up of natural polysaccharides are of interest due to non-toxicity and biodegradability. These polymers have hydrophilic tail and hydrophobic core for effectively encapsulation of hydrophobic drugs (Hsu et al., 2020; Liu, Du, & Zhai, 2015). BCS class II drugs poses a significant challenge for effective absorption in the therapeutic concentration. When these drugs are used in the treatment of cancer, parenteral administration is preferred; however, it does not go well with the patients. Biocompatibility and chemical modifiability of chitosan is advantageous for enhancing absorption of these drugs especially in the form of amphiphilic structure. In a research work, two compounds belonging to BCS class II, curcumin, an anti-inflammatory agent and 7-ethyl-10-hydroxycamptothecin, a cytotoxic agent, were individually conjugated with chitosan to form chitosan-drug amphiphile for enhancing GI absorption of the drugs for the treatment of colitis associated colorectal cancer (CRC). These novel formulations pre-clinically evaluated using CRC mouse model. Anti-inflammatory effect of curcumin nanoparticles was evaluated by using Raw 264.7 and murine bone marrow-derived cells. Pre-incubated cells with curcumin nanovesicles showed decreased secretion of pro-inflammatory cytokines, on the other hand, in case of CRC induced mice model; these nanoparticles reversed upregulation of ROS, which is involved in signal transduction and genomic instability, as compared to non-incubated cells. On the other hand, 7-ethyl-10-hydroxycamptothecin nanovesicles and free drug exhibited comparable anti-proliferation when evaluated in human colorectal DLD1 and HCT-116 cell lines, suggesting conjugation does not deter anti-proliferative activity of the drug. *Ex-vivo* near infrared imaging and confocal microscopy studies revealed accumulation of nanovesicles in the colon, as hypothesized by the authors. In DSS induced colitis mice model, authors reported that, administration of curcumin nanovesicles substantially reduced mice mortality, and typical colitis symptoms significantly. Similarly, these nanoparticles effectively targeted the tumor site, as revealed by tumor growth inhibition. Finally, low molecular weight chitosan platform made oral delivery of poorly aqueous soluble drugs possible and played vital role in drug targeting owing to its mucoadhesive potential, and negligible cytotoxicity (Han et al., 2019). Conventional therapies in the treatment of IBD (or other colonic conditions) lacks specificity, has poor bioavailability and retention and severe side effects upon long-term therapy. Administration by injectable route to overcome some of the above-mentioned lacunae, however, has poor patient compliance. Considering these factors oral nanotherapeutics is the emerging strategy. Chitosan-drug conjugates in amphiphilic form seems to bypass absorption from upper GIT and accumulates at the inflammatory/tumor sites, presumably due to reduced solubility of chitosan at acidic pH, increased molecular weight after conjugation and enhanced permeability and retention effect at the inflammation site.

Hydrophobic modification of chitosan by acetylation, or alkylation allows encapsulation of hydrophobic drugs, these drug molecules get physically embedded in the self-assembled nanocarriers and

Table 3
Examples of drug delivery systems developed by exploring interaction of chitosan with other natural polymers for the intestinal delivery of probiotics.

Polymer combined with chitosan for encapsulation	Probiotic encapsulated	Delivery system	Preparation technique	Reference
Sodium alginate	*Bifidobacterium breve*	Chitosan coated alginate microcapsules	External/ionic gelation, immersion coating	(Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2011)
	Lactobacillus plantarum	Chitosan coated alginate microcapsules	Electrospray	(Phuong Ta, Bujna, Kun, Charalampopoulos, & Khutoryanskiy, 2021)
	Lactobacillus casei 01	Cross-linked beads	Ionic gelation, polyelectrolyte complexation	(Ta et al., 2021)
	Bifidobacterium longum	Chitosan coated alginate microcapsules	Emulsification, internal gelation and immersion coating	(Ji et al., 2019)
	Lactobacillus plantarum 25	Chitosan-alginate microcapsules	Extrusion and cross-linking	(Jiang et al., 2013)
	Five strains of *Bifidobacterium* and *Lactobacillus*	Chitosan coated microcapsules prepared by	Emulsification, cross-linking and immersion coating	(Lohrasbi et al., 2020)
	Lactobacillus rhamnosus GG	Chitosan coated sodium alginate hydrogel particles	Extrusion and cross-linking	(Qi, Simsek, Ohm, Chen, & Rao, 2020)
	Bifidobacterium pseudocatenulatum G4	Chitosan coated microcapsules	Emulsification, internal gelation and immersion coating	(Kamalian, Mirhosseini, Mustafa, & Manap, 2014)
	Bacillus licheniformis	Chitosan hydrochloride-alginate micro-carriers	Polyelectrolyte complexation *via* orifice-polymerization	(Wu, Xu, Xie, Tong, & Chen, 2016)
	Escherichia coli strain Nissle 1917	Alginate coated chitosan microparticles	Layer-by-layer deposition by ionic gelation/polyelectrolyte complexation	(Luo et al., 2020)
	Lactobacillus gasseri and *Bifidobacterium bifidum*	Cross-linked beads	Ionic gelation/polyelectrolyte complexation	(Chávarri et al., 2010)
	Lactobacillus plantarum TN8	Chitosan coated alginate beads	Ionic gelation and immersion coating	(Trabelsi et al., 2013)
	Lactobacillus plantarum and *Bifidobacterium lactis*	Chitosan coated alginate microcapsules	Electro-hydrodynamic atomization, ionic gelation and immersion coating	(Zaeim, Sarabi-Jamab, Ghorani, & Kadkhodaee, 2019)
	Lactobacillus casei 01	Chitosan coated alginate microparticles	Spray-drying, ionic gelation, and polyelectrolyte complexation	(Ivanovska et al., 2017)
	Lactobacillus salivarius	Chitosan coated alginate beads	Emulsification, ionic gelation and immersion coating	(Youssef et al., 2021)
	Lactobacillus casei	Chitosan and carboxymethyl-chitosan coated beads	Ionic gelation, and immersion coating	(Li, Chen, Sun, Park, & Cha, 2011)
	Ligilactobacillus salivarius Li01	Carboxymethyl-chitosan and alginate microparticles	Layer-by-layer deposition, ionic gelation	(Yao et al., 2021)
	Bifidobacterium longum	Chitosan coated alginate microcapsules	Injection-gelation, immersion coating	(Yeung, Üçok, Tiani, McClements, & Sela, 2016)
	Bacillus coagulans	Chitosan-alginate microcapsules	Layer-by-layer deposition by polyelectrolyte complexation	(Anselmo, McHugh, Webster, Langer, & Jaklenec, 2016)
	Lactobacillus reuteri	Chitosan, thiolated chitosan coated alginate microcapsules	Emulsification ionic gelation, immersion coating	(Song Chen, Cao, Ferguson, Shu, & Garg, 2013)
	Bifidobacterium animalis	Microparticles	Atomization, ionic gelation, polyelectrolyte complexation	(Liserre, Ré, & Franco, 2007)
Alginate-starch	*Lactobacillus casei* and *Bifidobacterium bifidum*	Chitosan coated calcium alginate-gelatinized starch microcapsules	Emulsification ionic gelation, immersion coating	(Khosravi Zanjani, Tarzi, Sharifan, & Mohammadi, 2014)
Alginate-xanthan gum	*Lactobacillus plantarum*	Chitosan coated alginate-xanthan gum beads	Ionic gelation, and immersion coating	(Fareez, Lim, Mishra, & Ramasamy, 2015)
Starch	*Lactobacillus rhamnosus*	Chitosan- carboxymethyl high amylose starch tablets coated double-faced with carboxymethyl high amylose starch	Direct compression	(Calinescu & Mateescu, 2008)
Agar-gelatin	*Lactobacillus plantarum*	Chitosan coated agar-gelatin particles	Immersion coating	(Albadran, Monteagudo-Mera, Khutoryanskiy, & Charalampopoulos, 2020)
Pectin	*Lactobacillus casei*	Chitosan coated pectin microcapsules	Ionic gelation, immersion coating	(Bepeyeva et al., 2017)
Sodium alginate-pectin	*Lactobacillus acidophilus*	Chitosan coated pectin-alginate microbeads	Emulsification ionic gelation, immersion coating	(Odun-Ayo, Mellem, & Reddy, 2017)
Carboxymethyl cellulose	*Lactobacillus acidophilus*	Microcapsules	Layer-by-layer deposition by immersion	(Priya, Vijayalakshmi, & Raichur, 2011)
Dextran sulphate	*Lactobacillus acidophilus*	Hydrogels and beads	Polyelectrolyte complexation, cross-linking	(Yucel Falco, Falkman, Risbo, Cárdenas, & Medronho, 2017)
Xanthan gum	*Lactobacillus acidophilus*	Hydrogels and beads	Polyelectrolyte complexation	(Chen, Song, et al., 2015)

instantaneously dissolve when contacted with the GI fluids (Almeida et al., 2020; Kumar et al., 2020). Carboxymethyl chitosan (CMC), a water soluble derivative of chitosan has reported to increase activity of the drugs, therefore it has been explored extensively in the drug delivery systems to the colon (Vaghani, Patel, & Satish, 2012; Zhang et al., 2021). In the research work reported by Zheng H. and co-workers, water insoluble molecule 6-MP which is also one of the drugs used in the treatment of IBD is conjugated with CMC through disulphide bond for the hydrophobic modification of the polymer. Self-assembled carriers of this conjugate were anticipated to release the drug in the controlled

manner in the target cells as disulphide bond would reduce to free sulfhydryl group in response to the higher levels of glutathione present in the cytoplasm. Due to the lower pH in the intracellular environment (5.0–6.5), polymers can get shrunken and does not release the drug, even pH dependent polymers are not an exception to this, in contrast 6-MP-CMC conjugate released the drug at 10 mM concentration of Glutathione (GSH) at pH 5 as evident in the *in-vitro* dissolution studies (Zheng et al., 2011). Effective targeting to the colon is still a sought after drug delivery issue. Novel carrier mediated approaches seem to have prospects to address this issue due to their drug localization potential (Teruel et al., 2018; Turanlı & Acartürk, 2021; Wang, Han, et al., 2021). In monolithic dosage forms, combination of pH and time dependent release approach has been explored, but, with limited success (Patel, Shah, Amin, & Shah, 2009). Mesalamine, which is the first-line treatment for the IBD, rapidly gets absorbed from the small intestine as compared to the colon. In intestinal epithelial cells mesalamine undergoes rapid and extensive metabolism by the enzyme *N*-acetyltransferase to its *N*-acetyl-mesalamine derivative. This derivative is rapidly absorbed from the small intestine and may cause systemic toxicity and also reduces availability of mesalamine to the colon. Therefore, in one such research work, covalent interaction between EDTA and chitosan-EDTA (CH-EDTA) which resulted into a clear solution has been used as a polymer for coating of mesalamine tablet in a view to protect the drug release in the upper GIT. These coated tablets showed significant control over the release of the drug in *in-vitro* dissolution conditions mimicking stomach and small intestine as compared to eudragit coated marketed formulation of mesalamine. Due to permeation enhancing properties of chitosan, CH-EDTA coated tablets showed enhancement in bioavailability of mesalamine against uncoated ones and thereby achieved effective targeting to the colonic region/cells (Singh, Suri, Tiwary, & Rana, 2013). In another research work by Onishi and co-workers, succinyl-prednisolone was conjugated with chitosan as chitosan matrix alone was not able to control the release of the drug, later this conjugate was formulated into microspheres that were then coated with pH responsive polymer eudragit L-100. Due to small size below 10 μm micro- and nanoparticles retain better at the colitis sites than solid unit dosage forms. A combination of both pH and time controlled release was employed successfully to ameliorate 2,4,6-trinitrobenzenesulfonic acid-induced colitis in rat model (Onishi et al., 2008). Similarly, colon specific delivery of 5-ASA was achieved using N-succinyl chitosan matrices, which showed controlled release of the drug at acidic and alkaline pH against burst release observed at acidic pH through plain chitosan matrices (Mura et al., 2011).

5. Chitosan containing matrices in the delivery of probiotics to the colon

One of the causes of IBD is considered to be an imbalance between useful and harmful colonic bacteria and host-activated immune response against them (Lee & Chang, 2021; Prudhviraj et al., 2015). Probiotics are live microorganisms known to have several health benefits in humans when administered in adequate amounts. Apparently probiotics seems to be effective in the treatment of IBD due to their ability to stimulate anti-inflammatory cytokines, inhibition of pro-inflammatory cytokines, strengthening of the intestinal barrier function, and antagonistic action against pathogens (Asgari, Pourjavadi, Licht, Boisen, & Ajallouеian, 2020; Guandalini & Sansotta, 2019; Laroui et al., 2010). The major hurdle in the delivery of probiotics to the colon is the harsh acidic environment of the stomach where the bacteria may get killed if exposed (Dodoo, Wang, Basit, Stapleton, & Gaisford, 2017). Chitosan is an important polymer for the delivery of probiotics to the colon due to its ability to delay entry of acids and bile salts into the capsules *via* ion-exchange reactions due to formation of a thicker less porous membrane (Vaghani et al., 2012; Zhang et al., 2021; Zheng et al., 2011). Cross-linking of chitosan by anionic polymers and various chitosan derivatives are frequently used in the delivery of probiotics, related reports are summarized in Table 3.

6. Conclusion

There are five basic properties of chitosan, which are, 1. Biocompatibility, 2. Biodegradability especially by colonic microflora, 3. Cationic nature thereby enhanced mucoadhesion, and residence time at the inflamed colonic site, 4. Safety for long term administration due to GRAS status, and 5. Easy to modify chemical structure, underscores its utility for the colon targeted drug delivery. Multiparticulate dosage forms made up of chitosan and other natural polymers by cross-linking has shown pH dependent release of the payloads, hence they can protect sensitive molecules in the harsh acidic environment of the stomach. Structurally modified chitosan also has a potential to avert its dissolution at acidic pH. Therapy with proteins and peptides is the future of the treatment of the IBD, however suffers due to inappropriate route of administration, *i.e.* parenteral route and results into severe adverse effects; oral route certainly offers potential advantages. Delivery of proteins and peptides through chitosan based multiparticulate formulations achieve site specific drug release as well as localization into inflamed tissue. Targeting specific cells, such as macrophages and epithelial cells in the colon is a key therapeutic target, structurally modified chitosan based drug delivery systems offers formidable carrier for drug delivery to these targets. Chitosan based pellets using extrusion-spheronization can be formulated with ease due to good elastic properties of the polymer, which can be further coated with enteric polymers for the colon targeting. Due to sustained release properties of chitosan, better management of the IBD is possible. Chitosan itself has exhibited anti-inflammatory and immunomodulatory properties, it would be interesting if the polymer could produce synergistic/additive effect alongside therapeutic moieties in IBD. Finally, there is certainly a potential in this polymer for colon specific drug delivery, however, further studies are needed to prove commercial utility.

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