EXPERT OPINION

Chitosan: a potential polymer for colon-specific drug delivery system

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Introduction: There is an enormous growth and awareness of the potential applications of natural polymers for colon delivery of therapeutic bioactives. Chitosan (CH), a cationic polysaccharide, has a number of vital applications in the field of colon delivery and has attracted a great deal of attention from formulation scientists, academicians and environmentalists due to its unique properties.

Areas covered: CH has been widely explored for the delivery of drugs, peptides, proteins and genes to the colon for different therapeutic applications. Sustained and controlled delivery can be achieved with CH-based formulations like CH-coated tablets, capsules, beads, gels, microparticles and nanoparticles. This review mainly focuses on various aspects of CH-based formulations, particularly development of colon-specific delivery of drug.

Expert opinion: The vital properties of CH make it a versatile excipient, not only for sustained/controlled release applications but also as biodegradable, biocompatible, bioadhesive polymer. The colon is recognized as the preferred absorption site for orally administered protein and peptide drugs. The main problem associated with CH is limited solubility at higher pH due to reduced cationic nature, which also reduces mucoadhesiveness. The application of newer targeting moiety with CH-based formulations for highly site-specific delivery of bioactive has to be evaluated for further improvement of therapeutic index (bioavailability).

Keywords: chitosan, colon delivery, colon targeting, oral delivery

Expert Opin. Drug Deliv. (2012) 9(6):713-729

1. Introduction

Today, drug delivery researchers and formulation scientists have been directed toward the development and fabrication of dosage forms based on safe and effective biodegradable polymer, particularly of natural origin in pharmacology. Rouget described the deacetylated form of chitosan (CH) in 1859 [1]. During the last 20 years, a substantial amount of research has been published in relation to this polymer and its potential use in various applications. Recently, CH has found potential application in vast areas of drug delivery and tissue engineering due to its unique advantages and biological properties [2]. CH-based formulations or delivery systems have been widely studied for colonic drug delivery since it can protect therapeutic agents from the hostile environment of the upper gastrointestinal tract (GIT) and release the entrapped bioactive, specifically at the colon, through degradation of the glycosidic linkages of CH by colonic microflora [3]. The aim of this review is to provide an insight into the potential applications of CH as a pharmaceutical excipient for bioactive delivery to colon or colonic region.

CH is one of the most abundant polysaccharides found in nature, in fact second only to cellulose. Chitin may be obtained from arthropods/exoskeletons of

10.1517/17425247.2012.682148 © 2012 Informa UK, Ltd. ISSN 1742-5247

crustaceans such as crabs, shrimps, lobsters, fungi cell wall and insect shell (scorpions, spiders, cockroaches and silk worms) [4,5]. CH has a rigid crystalline structure through inter- and intramolecular hydrogen bonding [6,7].

CH (2-amino-2-deoxy-D-glucose) is a polycationic polymer which is obtained from chitin (β-linked *N*-acetyl-D-glucosamine) after its partial *N*-deacetylation and hydrolysis [3]. This linear aminopolysaccharide is composed of randomly distributed (1 → 4) linked D-glucosamine and *N*-acetyl-D-glucosamine units (**Figure 1**) [8,9]. The sugar backbone consists of β-1,4-linked D-glucosamine with a high degree of *N*-acetylation. The degree of deacetylation (DD) of commercial CH is usually between 70 and 95%, and the molecular weight (Mw) between 10 and 1000 kDa.

For the preparation of CH, ground shells are deproteinated and demineralized by sequential treatment with alkali and acid, after which the extracted chitin is deacetylated to CH by alkaline hydrolysis at high temperature [10]. Production of CH from these sources is inexpensive and easy. Further purification may be necessary to prepare medical and pharmaceutical grade CH. CH can be found in various forms differing in average Mw and DD and moreover the numerous chemical modifications that have been investigated. Except in certain fungi, CH is mainly present in the form of chitin in the nature. The production of CH from fungi, using fermentation methods is also gaining much interest in recent years.

2. Physicochemical properties of CH

2.1 Solubility

CH faces some limitations in its solubility depending on its molecular structure and pKa. This makes it necessary to dissolve chitin and CH in an appropriate solvent to reveal functionality. CH has limited solubility, that is, all acidic organic solvents (acetic acid (1 – 3%), tartaric acid and citric acid (4%) at pH less than 6.5 whereas chitin is insoluble in organic solvents [11]. The highly crystalline structure of CH enhances inter- and intramolecular hydrogen bonding which is responsible for limited solubility. However, chemical modifications and derivatization make it soluble in a wide range of pH conditions. Chung *et al.* in 2006 assayed the solubility, rheological and physico-chemical properties of CH and found that derivatives of CH exhibit a good solubility profile as compared with its native form [12]. In acidic solution it shows a cationic character due to the presence of primary amine groups on its backbone.

2.2 Degree of deacetylation and molecular weight

The Mw of CH is an important parameter which has to be considered during the development of formulation because it interferes with mechanical strength and barrier properties of the polymer. Park *et al.* studied the effect of different Mw CH and type of solvent on the properties of CH films and found that the tensile strength of film increased with CH Mw [13]. Acetic acid ensured the toughest films of CH followed by malic, lactic and citric acid, respectively. Tang *et al.* deliberated the effect of ultrasonication on the Mw and DD of CH and ascertained that the Mw and DD of CH nanoparticles decrease with an increase in the duration and amplitude of ultrasonication [14]. In another study, Huang *et al.* suggested that the Mw and DD of CH have a great effect on its transfection efficiency and uptake capacity when used in gene delivery [15].

2.3 Mucoadhesive character

CH is a natural bioadhesive polymer having an excellent mucoadhesiveness in swollen state that can adhere to hard and soft tissues. The adhesive properties of CH in a swollen state might involve some mechanisms like adhesion by hydration, hydrogen bonding and ionic interactions. Effective adhesion has been shown for epithelial tissues and in the mucus coat present on the surface of the tissues. When CH, having a polycationic surface (positively charged amino groups), interacts (electrostatically) with a mucin layer containing residues of sialic acid (negative charged monosaccharide), molecular attractive forces develop that help in the generation of mucoadhesive effect. A number of CH-based colloidal delivery systems have been published in the literature for the mucosal delivery of polar drugs, peptides, proteins, vaccines and DNA. It has been postulated that residence time of formulations at sites of drug action or absorption could be prolonged through the use of CH. It has also been intimated that CH might be valuable for drug delivery to specific regions of the GIT, that is, buccal mucosa [16,17], stomach [18,19], small intestine [20,21] and colon [22-24].

2.4 Biocompatibility

CH has been popular and marketed all over the world as a component in non-medical products, as a fat binder in cholesterol-lowering and -slimming formulations [25]. It has been proved that CH entraps lipids in the intestine due to its cationic nature [26,27]. CH is metabolized by certain human enzymes, especially lysozyme, and is considered biodegradable [28,29]. It has also been used in the biomedical field and has been found to be highly biocompatible [30]. Currently, CH has been approved by the authorities and a monograph relating to CH hydrochloride was included in the fourth edition of the European Pharmacopoeia (2002).

2.5 Biodegradation

Biometabolism in the body or biofate of polymers is an important aspect for the selection and use of any polymer in development of drug delivery systems. In the case of the systemic absorption of CH (hydrophilic polymers), it should have a suitable Mw for renal clearance. However, if the administered polymer size is larger than this, then it should undergo biodegradation (chemical or enzymatic) that would provide fragments suitable for renal clearance. Chemical degradation in this case refers to acid-catalyzed degradation bio-milieu, that is, in the stomach, at tumor site, or in endosome,

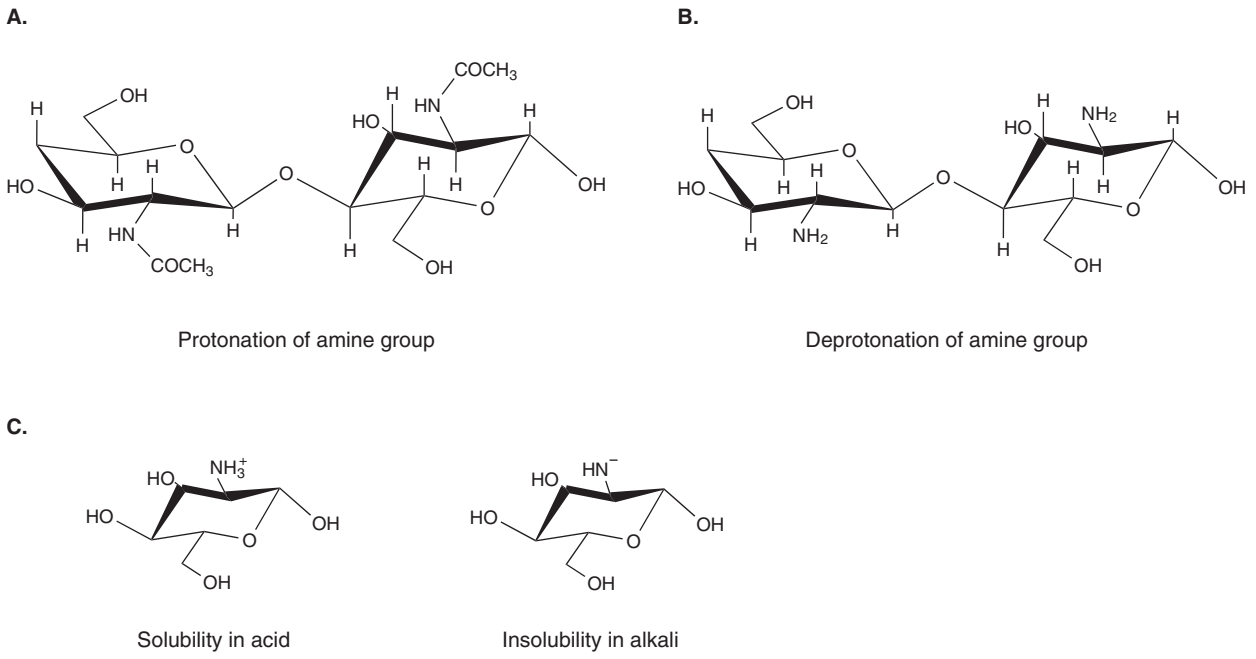

Figure 1. Chemistry of chitin (**A**), chitosan (**B**) and solubility of chitosan (**C**).

although a peculiar mechanism of *in vivo* degradation like oxidation–reduction depolymerization and free radical degradation have also been reported [31,32]. CH can be degraded by enzymes which hydrolyze glucosamine–glucosamine, *N*-acetyl-glucosamine–*N*-acetyl-glucosamine and glucosamine–*N*-ac etyl-glucosamine linkages [33]. CH is presumed to be degraded by lysozyme and by bacterial enzymes in vertebrates predominantly in the colon on oral administration [34]. In general, both rate and extent of CH biodegradability in living organisms depend on the DD. The CH degradation rate decreases with increasing DD [35,36]. As the extent of degradation is related to the rate, giving adequate time and appropriate conditions, the CHs would degrade sufficiently for subsequent excretion. The three enzymatically active forms of human chitinases are namely acidic mammalian chitinase (AMCase), di-*N*-acetylchitobiase and chitotriosidase identified have not been investigated with regard to the degradation of CH and/or its derivatives. AMCase was identified in the GIT and lung [37].

3. Colon-specific drug delivery

Colon-specific drug delivery has gained increasing attention in the treatment of colorectal cancer and other colon-related disorders, that is, Crohn's disease (CD), ulcerative colitis (UC), irritable bowel syndrome and spastic colon [38-40]. Some drugs, such as cardiovascular and antiasthmatic agents, have also been delivered via colon to avoid first-pass metabolism or the acidic environment of stomach. Colon targeting has also been proven very useful for systemic delivery of protein/peptide drugs because of the relatively low proteolytic activities in the colonic environment [41,42]. Drug delivery specifically to the colon can be achieved by oral or by rectal administration. Rectal delivery forms (suppositories and enemas) are not always effective and convenient because a high variability is observed in the distribution of drugs administered by this route. Suppositories are effective only in the rectum because of the confined spread and enema solutions can only be applied topically to treat diseases of the sigmoid and the descending colon. Therefore, the oral route is most preferred but absorption and degradation of the active ingredient in the upper part of the GIT is the major obstacle with the oral route and must be overcome for successful colonic drug delivery. There are several strategies currently followed for colon-targeted delivery, that is, prodrugs that become active at the colon, drug-eluting system responding to the pH, microflora-activated drug delivery systems, hydrogels and matrices and multicoating time-dependent delivery systems [39]. Absorption and degradation pathways in the upper GIT are the major obstacles in delivering drugs to colon. Hence, all the above strategies have attempted to prevent loss of the drug at the stomach and the small intestine, thereby facilitating quantitative drug delivery to the colon. For this purpose, it is very important to understand the pathophysiology of GIT.

3.1 General anatomical features of the colon

In terms of size and complexity, the human colon falls between that of carnivores such as the ferret, which has no identifiable junction between ileum and colon, and herbivores such as the rat which has a voluminous cecum [43]. The colon is made up of cecum, ascending colon, transverse colon, descending colon and rectosigmoid colon. It is approximately

Figure 2. Factors affecting drug absorption from colonic region.

1.5 m in length, with the transverse colon being the largest and the most mobile part [44].

The absorption of drugs from the colon depends on the rate of blood supply around the absorptive epithelium. The superior mesenteric artery provides arterial blood to the proximal colon whereas the inferior mesenteric artery supplies the distal colon. The superior (proximal colon) and inferior (distal colon) veins drain out the blood. The proximal part of colon receives a higher share of the blood flow than distal parts, although total colonic blood flow is less as compared with the small intestine [45,46].

The absorption capacity of the colon is less than that of the small intestine because of a lower surface area. The colon does not have any villi; however, the presence of plicae semilunares, which are crescentic folds, increases the intestinal surface of the colon to approximately 1300 cm^2. Despite the lower surface area, the colon has a large capacity for the absorption of water, electrolytes and short-chain fatty acids, which are formed during fermentation of carbohydrate, by the colonic bacteria [47]. The four vital functions of the colon are: i) to provide a suitable environment for the growth of colonic microorganisms such as *Bacteroides*, *Eubacterium* and *Enterobacteriaceae*; ii) reservoir for fecal contents; iii) expulsion of the contents from the colon at a suitable time and iv) absorption of water and sodium from the lumen, concentrating the fecal content, and secretion of potassium and bicarbonate. The factors that affect absorption from the colon are given in **Figure 2**. Colon-specific drug delivery is primarily dependent on two physiological factors, pH level and the transit time.

4. CH-based colon drug delivery systems

CH-based delivery systems have vital role in the colonic drug delivery since they can protect bioactives from the hostile conditions of the upper GIT and release the entrapped bioactive specifically at the colon through degradation of the glycosidic linkages of CH by microflora present in colon [3,22]. CH has been extensively used as a colon drug delivery polymer and a number of formulations are being developed for colon-specific drug delivery systems.

4.1 Tablets

Dozens of important research papers have been published on the use of CH as tablet excipient, that is, as a vehicle for sustained release tablets [48,49]; a direct compressible diluent [50-52]; a tablet disintegrant [53] and a tablet binder [54].

The versatile nature and biodegradable property of CH makes it a useful diluent in pharmaceutical preparations [55]. CH is also used as a coating material which is mainly useful for colon drug delivery due to its specific enzymatic degradation

Table 1. Summarized examples of some tablets based on CH for colon delivery of drug/bioactive.

Formulation	Drug	Preparation	Remarks	Ref.
Compression-coated tablets	Caffeine	Direct compression-CH and ethylcellulose used in the coat	CH level or the thickness of the coat can be varied to achieve a desired delivery profile	[120]
CH–CS interpolymer complex	5-FU	Compression coating technique using granulated CH	The X-ray imaging gave rise to the *in vivo* selectivity of this system for colon targeting	[121]
CH–CS-based matrix tablets	Budesonide	Tablets prepared by using Avicel pH 102 as diluent and Eudragit® L-100-55 as binder were coated to a weight gain of 10% w/w employing aqueous mixtures containing CH and CS	The aqueous CH/CS (40:60) coating could provide a facile method for delivering budesonide to the colon	[122]
Cationic polymethacrylate (Eudragit E-100)–mesalamine complexes	Mesalamine	Tablet core was coated with two thin layers, i.e., first CH coat for protection in the small intestine and second outer layer of Eudragit L-100 protect dissolution of the CH-covered core along GIT	In order to achieve a modulated drug release, carbomer P934 (1%) was also included. *In vitro/in vivo* correlations were not established	[123]
CH/pectin PEC	Vancomycin	Polyelectrolyte complexes between CH and pectin were prepared in various pH regions using different molar ratios by simple mixing. The precipitates were collected by spray-drying and tablets were obtained with the different complexes and vancomycin	CH/pectin complex prepared in molar ratio of 1:9 showed the highest mucoadhesiveness and a pH-dependent swelling sensitivity provide advantage for colon delivery	[124]

CH: Chitosan; CS: Chondroitin sulfate; 5-FU: 5-fluorouracil; GIT: Gastrointestinal tract; PEC: Polyelectrolyte complex.

property. Some of the examples of CH-based tablet formulation for colon delivery of drugs are described in **Table 1.**

Aiedeh and Taha synthesized a semisynthetic CH derivative by reacting CH separately with succinic and phthalic anhydrides for colon-specific drug delivery on oral administration [56]. The synthesized polymer was used in the fabrication of tablets and evaluated for *in vitro* dissolution studies which reveal that these tablets resisted dissolution under acidic conditions and showed improved drug release profiles under basic conditions. Hence, these polymers can be used for delivering drug to the colon. The matrix tablets of indomethacin was first developed by Amrutkar and Gattani for colonic delivery using novel cross-linked CH–chondroitin sulfate (CS) polyelectrolyte complex (PEC) [57]. They found the dissolution rate of the tablet depends on the concentration of polysaccharide used as binder and matrix, and time of cross-linking. They further concluded that a PEC of CH–CH might assist in improving colon drug delivery. Nunthanida *et al.* prepared amorphous spray-dried chitosan acetate (SD-CHA) and used as a binder in the preparation of theophyline granules which exhibited good flowability and an excellent compressibility profile [58]. They also observed the controlled drug release with the tablets prepared using SD-CHA as a tablet binder. These tablets exhibited Fickian diffusion control drug release. The release of drug from the CHA matrix tablets was at a minimum in an acidic environment (simulated gastric fluid (SGF)) and better release was observed in simulated intestinal fluid (SIF) and colonic fluid [59].

Macleod *et al.* investigated pectin- and CH-based PEC and conducted swelling studies [60]. They found minimal swelling occurring when the pectin:CH weight ratio was optimal for PEC formation, suggesting the formation of the PEC *in situ*. The study confirms the potential of pectin/CH/HPMC (hydroxypropylmethylcellulose) mixtures as a film-coating system for tablet cores, capable of achieving bimodal drug delivery and also triggering the drug release with colonic conditions. Kaur *et al.* formulated inter polymer complex films of CH and carboxymethyl tamarind kernel powder (CMTKP) for coating of budesonide tablets [61]. Tablets (Avicel pH 102 as diluent) coated to a weight gain of 10%, w/w with aqueous solutions of 40:60 or 50:50 ratio of CH:CMTKP did not release drug in pH 1.2 buffer. The histopathology of rat colon after oral administration of inter polymer complex film-coated tablets showed reduction in TNBS-induced (2,4,6-trinitrobenzene sulfonic acid) UC as compared with uncoated tablets. The tablets coated with 40:60 ratio of CH:CMTKP offer a great promise for colon delivery of budesonide, and providing drug concentration in the distal part of GIT for longer durations for effective therapy of inflammatory bowel disease (IBD).

Table 2. CH-based capsules for colon delivery.

Drug	Remarks	Ref.
Rebamipide	Absorption enhancers can increase the permeability of rebamipide across the colon tissue significantly and co-administration of C12 with rebamipide might also be very useful in local treatment for colon-specific diseases	[66]
Prednisolone	CH capsules might be useful for the colon-specific delivery of prednisolone and it enhances the effectiveness for the healing of colitis in rats with reduced side effects due to decreased intestinal transfer to the systemic circulation	[125]
ECT	The hypocalcemic effect started 6 – 8 h after oral administration of capsules and sustained for 24 h, which suggests that colon-specific delivery of ECT can be achieved using CH capsules and these additives may be useful for improving the colonic absorption of ECT in rats	[67]
CF	The CH capsules were effective for colon-specific delivery of a certain absorption enhancer and can improve their absorption enhancing action after oral administration. CH capsules may be useful carriers for colon-specific delivery of LM, thereby increasing its absorption-enhancing effect from the intestinal membranes	[65]

CH: Chitosan; CF: 5(6)-carboxyfluorescein; ECT: [Asu1,7]-eel calcitonin; LM: *n*-lauryl-β-D-maltopyranoside.

4.2 Capsule

Capsules made up of CH have gained attention for colon-specific delivery of peptide and anti-inflammatory drugs including insulin, 5-aminosalicylic acid (5-ASA) and ridogrel, and also to enhance intestinal absorption of insulin [22,61-63]. Tozaki *et al.* compared the healing effect of CH capsules containing a thromboxane synthase inhibitor (R68070) on UC induced by TNBS with that of a carboxymethylcellulose (CMC) suspension of R68070 [64]. It provided higher concentrations of R68070 in the large intestine as compared with CMC suspension which in turn provides a greater therapeutic effect against TNBS-induced UC than the CMC suspension. The same group examined the CH capsules for colon-specific delivery of 5-ASA. The surface of the CH capsules containing 5-ASA was coated with an enteric coating material (hydropropyl methylcellulose phthalate). The *in vivo* study was performed on TNBS-induced UC rats and found that the capsules were able to reach the large intestine 3.5 h after oral administration [63]. The myeloperoxidase (MPO) activities, the damage score and colon wet weight/body weight (C/B) ratio were measured to evaluate inflammation and colonic injury, and showed good results. CH capsule can also be used as potential carrier for colon delivery of absorption enhancers which could improve the absorption characteristics of the drugs [65,66]. The CH capsule has also been proved as a colonic absorption enhancer. Fetih *et al.* studied CH capsules for colon-specific delivery of [Asu1,7]-eel calcitonin (ECT) in rats [67]. The intestinal absorption of ECT was evaluated by plasma calcium levels after oral administration of the CH capsules containing ECT. The hypocalcemic effect started 6 – 8 h after oral administration of capsules and was sustained for 24 h which suggests that colon-specific delivery of ECT can be achieved using CH capsules and that the colonic absorption of ECT in rats can be improved. Tozaki *et al.* evaluated the intestinal absorption of insulin by measuring the plasma insulin levels and its hypoglycemic effects after oral administration of the developed CH capsules containing insulin [22]. The hypoglycemic effect started from 8 h after the administration of CH capsules. These findings suggest that CH capsules may be useful carriers for the colon-specific delivery of peptides including insulin. Some CH-based capsule formulations for colon delivery are described in **Table 2**.

4.3 Microparticles/microspheres

The potential advantages of CH in drug delivery include its ability to control the release of active agents and the avoidance of hazardous organic solvents while fabricating particles since it is soluble in aqueous acidic solution. Particulate drug carriers administered to mucosal surfaces of colonic region may protect the drug from degradation during the GIT passage, enhance the uptake by the epithelium and act as a controlled release system resulting in prolonged blood concentrations. The size and degradation rate depends on the method of preparation, types of CH or cross-linking methods [68,69]. There are a number of patents for CH microparticles prepared by SD-CHA (Mw 2392, 42,000, 142,000 Da) [70,71]. CH-based microparticulate carriers for colon drug delivery are listed in **Table 3**.

CH is also used to make beads [72,73] and for incorporation into liposomes [74] or alginate gel beads [75]. CH microparticles of prednisolone were prepared by a precipitation/coacervation technique using sodium sulfate as precipitant agent for colon delivery [76]. The microparticles were cross-linked using glutaraldehyde to increase their stability in the acidic environment of the stomach which may also result in a drastic decrease of bioadhesiveness and increased toxicity [77-79]. Lorenzo-Lamosa *et al.* described sodium diclofenac-loaded CH microparticles incorporated into Eudragit® polymers [80]. This approach resulted in controlled drug release using a dual approach, that is, pH-dependent and bacterial degradation of CH in the colonic environment. The double-coating system has been prepared and evaluated for colon delivery by Tominaga *et al.* [81]. They coated acetaminophen (core) with an inner coating layer made of CH and then outer coating layer made of phytin (gastric acid-resistant material protects the core from the acidic environment

Table 3. CH-based microparticulate carriers for colon-specific delivery.

Method of preparation	Drug	Purpose	Remark	Ref.
Emulsion cross-linking	DTZ	Chronotherapy of chronic stable angina	Exploiting the properties of enzymatic degradation of CH and pH-dependent solubility of Eudragit® S-100	[126]
	Aceclofenac	Rheumatoid arthritis	Release kinetics data were best fitted with the Higuchi model and showed zero-order release with non-Fickian diffusion mechanism	[127]
	5-ASA and camylofine dihydrochloride	UC	Orally administered microspheres of both drugs can be used together for the specific delivery of drug to the colon and reduce symptoms of UC	[128]
Emulsion cross-linking method followed by microencapsulation with Eudragit S-100 by solvent evaporation technique	Ondansetron	Multiparticulate system containing CH microspheres for the treatment of irritable bowel syndrome	Drug release followed Peppas model	[129]
CH as core polymer and entrapped within Eudragit S-100	Valdecoxib	Colorectal cancer	Drug release at pH 7.4 and good mucoadhesive property of the microspheres proved potential for colonic delivery	[130]
Ca-alginate beads encapsulating a probiotic and prebiotic followed by coating of CH	Probiotic and prebiotic	For enhancing survival of the probiotic bacteria and keeping intact the prebiotic during exposure to the adverse conditions of the GIT	The microencapsulation of *Lactobacillus gasseri* and *Bifidobacterium bifidum* with alginate and a CH coating offers an effective means of delivery of viable bacterial cells to the colon and maintaining their survival during simulated gastric and intestinal juice	[131]
Emulsion-complex coacervation	Ovalbumin	CS-CH for oral proteins delivery	1:1 CS:CH ratio suitable for a possible colon targeting on the basis of ovalbumin release profile	[132]
One-step spray-drying technique	5-FU	Wheat germ agglutinin -conjugated CH-Ca-alginate microparticles for colon cancer	microparticles showed excessive mucoadhesiveness *in vitro*, due to the positive surface charge, pH-dependent swelling of the matrix and lectin-sugar recognition	[133]
Cross-linked polyelectrolyte microparticles	Budesonide	A targeted delivery system for IBD	CH-Ca-alginate microparticles efficiently loaded with budesonide were designed using one-step spray-drying process followed by Eudragit-coating	[134]

5-ASA: 5-aminosalicylic acid; CH: Chitosan; CS: Chondroitin sulfate; DTZ: Diltiazem hydrochloride; 5-FU: 5-fluorouracil; GIT: Gastrointestinal tract; IBD: Inflammatory bowel disease; UC: Ulcerative colitis.

of stomach). The inner layer of CH protected the core in the small intestine and was then degraded in the colon via enzymatic degradation where the drug was released. The authors developed a multiparticulate system by coating cross-linked CH microspheres with combination of Eudragit L-100 and S-100 as pH-sensitive polymers, for colon-specific delivery of metronidazole. *In vitro* drug-release studies were performed in a simulating environment of stomach-to-colon transit in the presence and absence of rat cecal contents. The results showed a pH-dependent release of the drug attributable to the presence of the Eudragit coating. Moreover, the drug release was found to be higher in the presence of rat cecal contents, indicating the susceptibility of the CH matrix to colonic enzymes [38].

The CH–Ca-alginate microparticles were also developed using the spray-drying method, followed by ionotropic gelation/PEC for colonic delivery of 5-ASA [82]. They showed controlled release rate of drug *in vitro* and localization of 5-ASA in the colon with lower systemic bioavailability.

4.4 Beads

Beads are multiparticulate dosage forms which have been extensively investigated for oral drug delivery, served as depot reservoir and allow the sustained release of bioactives. The hydrogel beads of CH were prepared for extended release of verapamil (VP) by tripolyphosphate (TPP) and further cross-linked using glutaraldehyde. The beads were evaluated for physical properties such as size, shape, friability and loading efficiency. They found spherical geometry of beads (mean diameter range 1.3 – 2.0 mm) with 42% drug-loading efficiency with less than 1% friability indicating that the beads surfaces are highly resistant to attrition. The beads of medium Mw CH showed slowest release rate with good floating characteristics and long buoyancy (more than 6 h).

The alginate-chitosan (ALG-CH) blend gel beads based on dual cross-linking were developed by Xu *et al.* which protect the bioactive under GIT conditions [83]. The sustained release profiles of single and dual cross-linked gel beads-loaded bovine serum albumin (BSA) were observed in SGF, SIF and simulated colonic fluid (SCF), suggesting the dual cross-linked beads have potential to deliver bioactives to the small intestine or colon. CH hydrogel beads for colon-targeted delivery of satranidazole were prepared by chemical cross-linking, followed by enteric coating with Eudragit S-100 and exhibited that Eudragit S-100 coating on the CH beads prevented premature drug release in simulated upper gastrointestinal (GI) conditions and most of the loaded drug was released in the colon, an environment rich in bacterial enzymes that degrade the CH [23].

Single-, double- or multilayered beads of ciprofloxacin were prepared by simple ionic cross-linking with sodium TPP and coated with alginate and/or CH [84]. The number of coatings or thickness of coat on beads influenced release rate of ciprofloxacin, beads having three coatings, viz., alginate, CH and finally alginate, the release appeared to follow the pattern suitable for colon-targeting, that is, time-dependent release behavior.

4.5 Nanoparticles

CH nanoparticles have been contrived using several techniques such as ionotropic gelation, emulsification, solvent evaporation, reverse micellar, spray-drying, coacervation and sieving methods [85-87]. The colonic delivery potentials of CH-based nano-formulations are listed in **Table 4**. The preparation technique and physicochemical property of drugs have been mainly responsible for drug-loading efficiency of any carrier. Elzatahry and Eldin prepared metronidazole bearing CH nanoparticales for colonic delivery [88]. Hyaluronic acid (HA)-coupled CH nanoparticles of 5-fluorouracil (5-FU) were prepared using an ionotropic gelation method for targeted delivery to the colon tumors [89]. The HA-coupled CH nanoparticles showed enhanced cellular uptake by HT-29 colon cancer cells compared with the uncoupled CH nanoparticles. The cytotoxicity of 5-FU incorporated in HA-coupled CH nanoparticles was higher compared with the free 5-FU solution.

Li *et al.* synthesized folate-chitosan (FA-CH) conjugates by coupling FA with CH based on chemical linking of carboxylic group of FA with amino group of CH and confirmed by Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (H NMR) [90]. The FA-CH nanoparticles prepared by ionic gelation method using sodium TPP were positively charged with a spherical shape and a particle size of about 100 nm. The improved cellular uptake of CH or FA-CH nanoparticles in HT-29 colon cancer cells was observed through fluorescent microscopy and showed a potential targeted drug delivery system for colorectal cancer.

Venkatesan *et al.* developed CH-modified hydroxyapatite nanocarriers to deliver celecoxib for colon cancer [91]. The effects of celecoxib-loaded nanoparticles on colon cancer cell proliferation, morphology, cytoskeleton, cellular uptake and apoptosis were analyzed *in vitro* and the researchers found significant antiproliferation, apoptosis and time-dependent cytoplasmic uptake of celecoxib-loaded CH-modified hydroxyapatite nanoparticles in HCT-15 and HT-29 colon cancer cells. The *in vivo* studies, that is, antiproliferative, apoptotic and tumor inhibitory efficacy of celecoxib-loaded nanocarriers were performed in a nude mouse human xenograft model and showed significantly greater inhibition of tumor growth following treatment with this modified nanoparticle system.

Tahara *et al.* developed decoy oligonucleotide (ODN)-loaded CH-modified poly(D,L-lactide-co-glycolide) (PLGA) nanospheres (NS) by emulsion solvent diffusion method and evaluated for UC [92]. CH-modified PLGA NS showed positive zeta potential, while unmodified PLGA NS was negatively charged. The uptake studies were performed on Caco-2 cells using confocal laser scanning microscopy (CLSM) indicated that CH-PLGA NS were more effectively taken up by the cells than plain-PLGA NS. *In vivo* study suggested decoy ODN-loaded CH-PLGA NS were specifically deposited and adsorbed on the inflamed mucosal tissue of the UC model rat.

4.6 Hydrogels

Hydrogels are cross-linked polymeric networks that have a high number of hydrophilic domains and absorb large amount of water or biological fluid which can dramatically increase their volumes [93,94]. CH hydrogels have been used for the delivery of macromolecular compounds, such as peptides, proteins, antigens, ODNs and genes. The ionic, polyelectrolyte, interpolymer complex and hydrophobic associations are four major physical interactions that lead to the gelation of a CH solution. CH hydrogels have great potential as oral drug delivery system as they possess pH-sensitive or enzyme-specific release. CH-based PEC hydrogels have been explicated to bypass the acidic environment of the stomach and release the loaded drug into the desired site (intestine). Hari *et al.* explicated nitrofurantoin-loaded CH-alginate hydrogel microcapsule and showed selective drug release in an intestinal medium compared with a gastric medium (acidic environment), due to the pH-dependent swelling properties

Table 4. Colonic delivery potential of CH-based nano formulations.

Carrier/polymer	Drug/bioactive	Methodology	Remarks	Ref.
FA-CH conjugated nanoparticles		Cross-linking FA-CH conjugates with Na TPP	Nanoparticles showed improved uptake in HT-29 cell and could become a potential targeted drug delivery system for colorectal cancer	[90]
CH-CMS nanoparticles	5-ASA	Complex coacervation process under mild conditions	The release of 5-ASA from nanoparticle was based on the ion-exchange mechanism	[135]
CH-modified NS	NF-κB decoy ODN	Emulsion solvent diffusion method	Uptake studies with Caco-2 cells using CLSM and *in vivo* studies exhibited CH-PLGA NS provide oral decoy ODN delivery in UC	[92]
Hydroxyapatite-CH nanocomposite	Celecoxib	Coprecipitation method	The *in vivo* human colon tumor xenograft nude mouse tumor studies proved the celecoxib-loaded Hap-Cht nanoparticles were more potent in inhibiting tumor growth than free celecoxib and not elicit any serious side effect	[93]
Thiolated CH-coated PMMA nanoparticles	Paclitaxel	Nanoparticles were synthesized through radical polymerization of methyl methacrylate initiated by cerium (i.v.) ammonium nitrate and coated by CH-glutathione conjugate	The paclitaxel-loaded nanoparticles showed cytotoxicity for NIH 3T3 and T47D breast carcinoma cells, along with no cytotoxicity for two colon cell lines (HT-29, Caco-2) and showed sustained *in vitro* drug release	[136]
Nano-complex based on gal-LMWC	ASO	Gal-LMWC associated with ASO to form a stable nano-complex	A non-viral gene vector was combined with an ASO and targeted to activated macrophages for treatment of CD. The inhibition of TNF-α by this strategy represents a promising therapeutic approach for the treatment of CD	[137]
CH–DNA nanoparticles	DNA	Ionotropic gelation method	CH–DNA nanoparticles were more stable in the upper regions of the small intestine suggested that higher uptake rates may occur in the duodenum compared with the ileum and the colon	[138]
Eudragit® S-100-coated pellets bearing HA-coupled CH nanoparticles	Oxaliplatin	HA-coupled CH nanoparticles bearing oxaliplatin prepared by ionotropic gelation then formed pellets and coated with Eudragit S-100	*In vivo* study on tumor-bearing Balb/c mice showed targeting potential of HA-coupled CH nanoparticles to the colon and tumor	[139]
Nanoparticles	5-ALA	Ionic gelation method	CH nanoparticles could exclude the influence of normal flora inside the gut and serves as an adequate tool for fluorescent endoscopic detection of colorectal cancer cells *in vivo*	[140]

5-ALA: 5-aminolevulinic acid; 5-ASA: 5-aminosalicylic acid; ASO: Antisense oligonucleotide; ATRA: All-trans retinoic acid; CD: Crohn's disease; CH: Chitosan; CLSM: Confocal laser scanning microscopy; CMS: Carboxymethyl starch; FA: folate; 5-FU: 5-fluorouracil; gal-LMWC: Galactosylated low molecular weight CH; HA: Hyaluronic acid; MPEG: Methoxy poly(ethylene glycol); NF-κB: Nuclear factor kappa B; NS: Nanospheres; ODN: Oligonucleotide; PEC: Polyelectrolyte complex; PLGA: Poly(D,L-lactide-co-glycolide); PMMA: Poly(methyl methacrylate); TPP: Tripolyphosphate; UC: Ulcerative colitis.

Table 4. Colonic delivery potential of CH-based nano formulations (continued).

Carrier/polymer	Drug/bioactive	Methodology	Remarks	Ref.
Nanoparticles of quaternized CH derivatives	Insulin	PEC method	*ex vivo* studies revealed better insulin transport across the colon membrane of rats for nanoparticles made with quaternized derivatives and *in vivo* studies showed enhanced colon absorption of insulin in diabetic rats	[141]
MPEG-grafted CH nanoparticles	ATRA	Ion-complex formation between ATRA and CH	The apoptosis was progressed on treatment with ATRA bearing MPEG-grafted CH nanoparticle	[142]
HA-coupled CH nanoparticles	5-FU	PEC method	5-FU in HA-coupled CH nanoparticles was about 2.60-fold more effective than free 5-FU on HT-29 cells	[89]

5-ALA: 5-aminolevulinic acid; 5-ASA: 5-aminosalicylic acid; ASO: Antisense oligonucleotide; ATRA: All-trans retinoic acid; CD: Crohn's disease; CH: Chitosan; CLSM: Confocal laser scanning microscopy; CMS: Carboxymethyl starch; FA: folate; 5-FU: 5-fluorouracil; gal-LMWC: Galactosylated low molecular weight CH; HA: Hyaluronic acid; MPEG: Methoxy poly(ethylene glycol); NF-κB: Nuclear factor kappa B; NS: Nanospheres; ODN: Oligonucleotide; PEC: Polyelectrolyte complex; PLGA: Poly(D,L-lactide-co-glycolide); PMMA: Poly(methyl methacrylate); TPP: Tripolyphosphate; UC: Ulcerative colitis.

of the PEC hydrogel [95]. The CH hydrogel protects the peptide from degradation by intestinal peptidases by the attachment of enzyme inhibitors to the CH polymer. The serine proteases are suppressed by the covalent attachment of competitive inhibitors (i.e., Bowman–Birk inhibitor) and metallo-peptidases are inhibited by CH derivatives displaying complexing properties, that is, CH–EDTA (ethylenediaminetetraacetic acid) conjugates [96]. The large intestine is considered a safe absorption site for orally delivered peptides and proteins as proteolytic activity is lower in the colonic region than in the small intestine. Mesalazine (5-ASA), acetaminophen, sodium diclofenac and insulin showed good results and higher uptake within the colon when loaded in CH-based hydrogel [97,67,63,22].

4.7 Vaccine delivery

The oral nanoparticles delivery is thought to have the potential to provide mucosal protective immune responses, one of the most important goals of modern vaccinology. The oral route is a more convenient route of administration for vaccines with Peyer's patches as the main target. The vaccine is protected against enzymatic degradation via incorporating it into a carrier system and CH is the best suitable polymer, able to open the tight junctions and allows paracellular transport across the epithelium. The soluble CH formulations are able to open the tight junctions, whereas particulate vaccine delivery systems are taken up by the M-cells and subsequently biodegraded. The submicron size of nanoparticles easily allows them to be taken up by M-cells, in mucosa-associated lymphoid tissue (MALT), that is, gut-associated lymphoid tissue initiating site of vigorous immunological responses. Roy *et al.* successfully loaded plasmid pCMVArah2 encoding peanut allergen gene into CH nanoparticle with good antigen expression and protection results after oral administration in mice [98].

4.8 Protein peptide delivery

The pharmaceutical industry and practitioners involved in the health system are interested in the development of oral peptide delivery systems because oral formulations for therapeutic peptides prognosticate the greatest ease of application and a high patient compliance, and also excluding any risks such as infections caused by non-sterile needles or hemolytic effects. The efficacy of oral formulations, however, is limited by different barriers encountered with the GIT, that is, absorption [99] and enzymatic barrier [100] which are mainly responsible for a very low bioavailability of orally given peptides and proteins. The colon is the most preferred absorption site for orally administered protein and peptide drugs because of the relatively low proteolytic enzyme activity in it. CH and its derivatives provide new vistas in peroral peptide delivery systems as they are able to reduce both barriers. For the first time ever, in 1994, Illum and Farraj showed the permeation-enhancing capability of CH [101]. CH is useful for paracellular transport across the epithelium because it may be able to open the tight junctions which is important for the transport of hydrophilic compounds such as peptides and proteins across the membrane. An *in vitro* study with Caco-2 cell monolayers ascertained a significant reduction in the transepithelial electrical resistance (TER) after the addition of CH [102]. The positive surface charge of CH interacts with the cell membrane resulting in a structural reorganization of tight junction-associated proteins, thus explicating the mechanism underlying the permeation-enhancing effect [103]. CH with a high DD and with a high molecular mass exhibits a comparatively greater epithelial permeability.

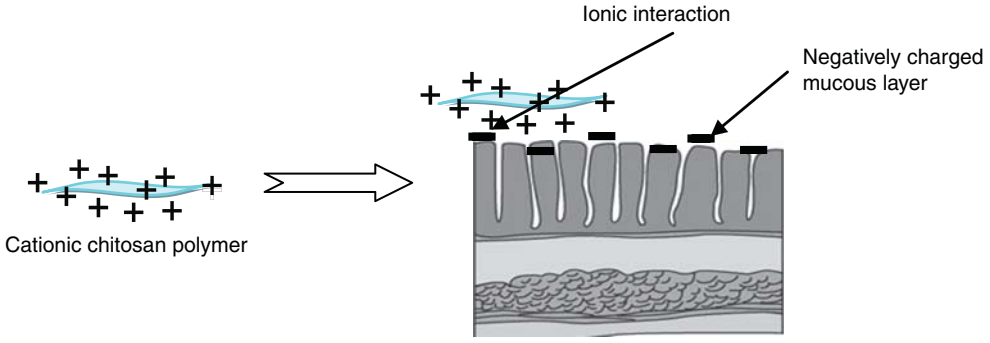

Figure 3. Interaction of chitosan with mucous layer.

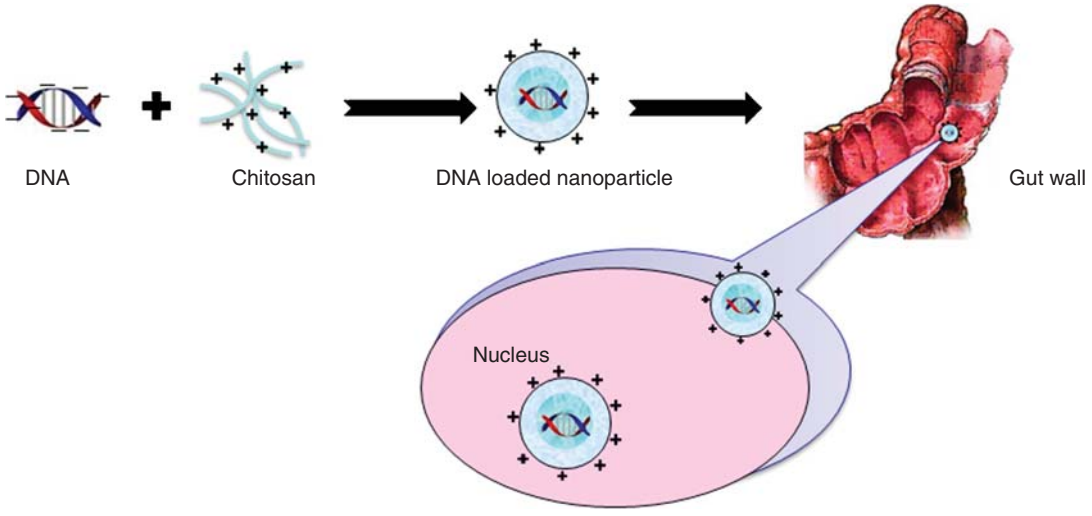

Figure 4. Cellular internalization of chitosan–DNA complex.

CH significantly increased the transport of buserelin, 9-desglycinamide 8-L-arginine vasopressin and insulin in Caco-2 cell monolayers [104,105]. CH derivatives have been developed to improve its quality, such as glutamate, aspartate and hydrochloride salts which have been used for colon-specific drug delivery and to enhance the delivery of therapeutic peptide across intestinal epithelia [105-107]. The permeation-enhancing effect of CH is comparatively reduced in the presence of the mucus layer, because it cannot reach the epithelium due to size limited diffusion and/or competitive charge interactions with mucins [108].

4.9 Gene delivery

In 1995, Mumper *et al*. first proposed CH as a potential gene delivery vector [109]. The cationic charged CH interacts electrostatically with negatively charged DNA and forms PEC in which the DNA becomes better protected against nuclease degradation. Mao *et al.* developed DNA–CH NP by a complex coacervation method using sodium sulfate as desolvating agent and yielding particles in the size ranging from 200 to 500 nm [110]. Jayakumar *et al.* developed small interfering RNA (siRNA) entrapped CH–TPP nanoparticles and found high binding capacity and loading efficiency, which showed better vectors for the silencing effect in CHO K1 and HEK 293 cells [111]. Tahara *et al.* reported that CH-modified PLGA NP could increase cellular uptake of Cy3-labeled siRNA into A549 cells [112]. Lee *et al.* developed CH/polyguluronate (PG) nanoparticles for siRNA delivery and found low cytotoxicity with ability to transport siRNA into cells [113]. Recently, CH/siRNA nanoparticles have been used as aerosolized formulation for gene silencing in transgenic EGFP mouse lungs [114]. On the bases of recent research, it is evident that CH and its derivatives have enormous properties in terms of DNA loading, condensation and intracellular gene delivery.

5. Mechanisms of action of CH on epithelial cell or colonic mucosa

As studied by many researchers, CH is being used extensively as an *in vitro* and *in vivo* enhancer for transmucosal drug delivery, but still it's difficult to understand mechanisms of

action on mucosal epithelium. The mechanism of action of CH could depend on a combination of mucoadhesion and an effect on tight-junction (TJ) regulation [102]. The mechanism of CH nanoparticle transport across the GIT is most probably through adsorptive endocytosis. Electrostatic interaction between positively charged CH and negatively charged sialic acid of mucin facilitates the association of CH nanoparticle to the mucus layer and subsequent internalization via endocytosis (**Figure 3**) [115,116]. TER denotes epithelial permeability, which is inversely proportional to the permeability of the epithelial layer to organic ions and CH's effects are concentration-dependent and reversible. Reversible effects of CH on Caco-2 cell line were observed indicating that it acts temporarily on the cellular barrier [117]. The CH-induced redistribution of F-actin has been shown to be important in regulating paracellular flow across cultured intestinal epithelia, and the described effects of CH on epithelial barrier function might result, at least partially, from alterations of the cytoskeleton [118]. The modification of membrane integrity was confirmed by cell line studies performed using fluorescent, cell-impermeant probes of different Mw [119]. These experiments demonstrated that the intracellular appearance of the probe was dependent on its Mw.

6. Expert opinion

CH is a polymer of choice for the development of colon-specific drug delivery systems and potential biomedical applications have gained the attention of formulation scientists. Considerable efforts have been devoted to the development of CH-based drug delivery systems which can effectively and specifically deliver therapeutics at the colon-site via the oral route. PubMed indexed more than 2000 articles related to CH in the year 2011, which reflects that CH is a potential and active polymer for future therapeutic applications. For instance, CH is useful for oral gene delivery due to its adhesive and transport-enhancing properties in the gut. CH forms complexes with DNA, can facilitate transfection and inhibits degradation of DNA (**Figure 4**). As discussed in the review, the vital properties of CH make it a versatile excipient, not only for sustained/controlled release applications but also as biodegradable, biocompatible and bioadhesive polymer.

CH degradation or digestion is affected by enzymes released from colonic bacteria which hydrolyze glucosamine–glucosamine, *N*-acetyl-glucosamine–*N*-acetyl-glucosamine and glucosamine–*N*-acetyl-glucosamine linkages, which triggers the release of controlled bioactives from CH-based formulations (i.e., nanoparticle, microspheres, microparticle). However, existing evidence for the efficacy of these drug carriers lacks consistency, partly due to the large diversity of CH sources and methodologies used for their preparation. The main problem associated with CH is its limited solubility at higher pH due to reduced cationic nature, which also reduces mucoadhesiveness. The highly cationic nature of CH makes it toxic to negatively charged cells which may be due to disruption of the cell wall at a higher dose of CH. CH and its derivatives seem to be toxic to several pathogens, that is, bacteria, fungi and parasites which could be useful in infectious diseases. The limited solubility of CH in neutral and basic aqueous media limits its utility but with the help of chemical modifications, that is, derivatization, it can be solubilized at both neutral and basic pH. The derivatization or chemical modifications can also be used to execute a hydrophobic, cationic and anionic property which has opened new era for its versatile applications in various fields. Further studies on the chemical and enzymatic modification of commercially available CH is aimed at tailoring their physical and chemical properties, functionality and bioavailability for different therapeutics, which are essential in developing more dedicated and effective delivery systems for the release of protein and polypeptide drugs to the colon site.

Colonic membrane permeability is limited to water and potassium ions due to the presence of various barriers. Hence, for systemic absorption of bioactives/therapeutic moieties across the colonic membranes, permeation enhancers are required with a colon-specific drug delivery system. The use of CH in the design of a colon-specific drug delivery system overcomes this problem as it has properties to enhance the colonic membrane permeability. Moreover, due to the presence of more reactive groups in the structure of CH, the surface of nanoparticle/microparticles developed using CH can be modified by attaching highly site-specific moieties and can be used to deliver anticancer drugs specifically to colorectal cancer. CH can also be utilized in vaccine development wherein the polymer will act along with the antigen/peptide to result in eliciting a marked and increased immunogenic response. CH has made its presence in the pharmaceutical and biomedical applications but efforts must be arranged and entire biomedical fraternity should work toward the launch of CH-based potential drug delivery system in the market so that mankind can benefit.

Declaration of interest

The authors state no conflict of interest and have received no payment for the preparation of this manuscript. A Gulbake is thankful to RGNF-UGC New Delhi, India for providing financial assistance to carry out the project on colon drug delivery.

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