Bacteria aided biopolymers as carriers for colon specific drug delivery system: A Review

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Abstract: Biopolymers are promising materials in the delivery of drugs due to their compatibility, degradation behaviour, and nontoxic nature on administration. On suitable chemical modification, these polymers can provide better materials for drug delivery systems. Biopolymers like natural polysaccharides obtained from various sources are being extensively used for the development of solid dosage forms for delivery of drug to the colon. The rationale for the development of a biomaterial based drug delivery system for colon is the presence of large amounts of polysaccharidases in the human colon as the colon is inhabited by a large number and variety of bacteria which secrete many enzymes e.g. β-D-glucosidase, β-D-galactosidase, amylase, pectinase, xylanase, β-D-xylosidase, dextranase, etc.. A large number of polysaccharides have already been studied for their potential as colon-specific drug carrier systems, such as the polysaccharides, from algal origin (e.g. alginates), plant origin (e.g. pectin, guar gum, locust bean gum, khaya gum, konjac glucomannan) microbial origin (e.g. dextran, xanthan gum) and animal origin (Chitosan, chondroitin, hyaluronic acid). The ability of these natural polysaccharides to act as substrates for the bacterial inhabitants of the colon together with their properties, such as swelling and film forming has appeal to area of colon specific drug delivery as it is comprised of polymer with large number of derivatizable groups, with wide range of molecular weight, varying chemical composition, biocompatibility, low toxicity and biodegradability and a high stability. Various major approaches utilizing biopolymers in modified or unmodified form, for colon-specific delivery like fermentable coating of the drug core, embedding of the drug in biodegradable matrix and multiparticulate formulation of drug-saccharide conjugate (prodrugs) are discussed.

Keywords: Colon specific drug delivery system, biopolymer, polysaccharides.

INTRODUCTION

The ability to deliver drugs to the human colon in a specific manner has become feasible in the last decade. The necessity and advantages of colon specific drug delivery systems have been well recognized and documented. Colonic targeting has gained increasing interest over the past years. A considerable number of publications dealing with colon targeting, colon-specific drug delivery, and absorption from the caecum and other colon sections indicate a growing focus of research activities in this area. Targeted delivery of the drugs to the colon has attracted much interest for treatments of local diseases of the colon like ulcerative colitis, Crohn’s disease and colon cancer. Generally colon is not as
suitable a site for drug absorption as is the small intestine, because water content in the colon is much lower and the colonic surface area for drug absorption is narrow in comparison with the small intestine. The colonic region however is recognized as a preferable site of absorption of protein and peptide drugs, because of less hostile environment prevailing in the colon compared with stomach and small intestine. Also Colon-specific drug delivery can improve the efficacy and reduce side effects by exerting high drug concentrations topically at the disease site. Drugs that are destroyed by the digestive enzymes and metabolized by pancreatic enzymes are minimally affected in the colon. \([1]\) For treatments of colon cancer, drug targeting not only reduces the dose to be administered, but also reduces the incidence of possible adverse effects associated with these chemotherapeutic agents.

Based on literature review, the following different categories of drugs are suitable for colon specific drug delivery.

1. Drugs used to treat irritable bowel disease (IBD) require local delivery of drug to colon e.g. sulphasalazine, Olsalazine, Mesalazine, steroids like Fludrocortisone, Budesonide, Prednisolone and Dexamethasone.

2. Drugs to treat colonic cancer e.g. 5-fluorouracil, doxorubicin, and methotrexate.

3. Protein and peptide drugs - Proteins, peptides and oligonucleotides composed of large molecules can be easily absorbed through the gastrointestinal mucosal wall.\([2]\) The colon is also known to be rich in lymphoid tissues where ingestion of antigens into the colonic mucosa stimulates the rapid production of antibodies, thus promoting this route as a means for vaccine delivery.\([3]\) e.g. Growth hormones, Calcitonin, Insulin, Interleukin, Interferon and Erythropoietin.

4. Colon targeted drug delivery could be used to achieve chronotherapy for diseases that are susceptible to circadian rhythms, in other words they have peak symptoms in the early morning, such as nocturnal asthma, angina, hypertension and arthritis. \([4-6]\)

5. Some lipophilic vitamins, as well as bile salts and some steroids that undergo enterohepatic circulation have been known to show satisfactory colonic absorption. \([7]\)

Colon specific drug delivery system relies on exploiting a unique feature of the intended site and protecting the active agent until it reaches that site. \([8]\) Colonic drug delivery may be achieved by either oral or rectal administration. Rectal dosage forms (enemas and suppositories), are not always much effective due to high variability in the distribution of drug administered by this route and the drug do not always reach the specific sites of the colonic diseases and the sites of colonic absorption. \([9,10]\) To deliver the drug in colon, the last part of the gastrointestinal tract, it must first of all pass through the stomach, the upper part of the intestine and must use the characteristics of the colon to specifically release the drugs in this part of the digestive tract. Because of the distal location of the colon in the gastrointestinal (GI) tract, an ideal colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and affect an abrupt onset of drug release upon entry into the colon. This requires a triggering mechanism built in the delivery system responsive to the physiological changes particular to the colon. However, the physiological similarity between distal small intestine and the proximal colon presents very limited options in selecting an appropriate drug release triggering mechanism.

Since it is virtually impossible to treat the ascending colon via the rectum, oral treatment is the only reliable method of delivery. Currently, several strategies are being used for targeting the drug specifically to the colon viz. systems that are, pH dependant, pressure controlled, prodrugs, bioadhesion, colon microflora activated and those based on biodegradable polymers (enzyme controlled).\([11-13]\) Colonic delivery via the oral route requires control of four factors: time of release, site of release, extent of dispersion, and modification of low flux across the absorptive epithelium. Certain components provide specific mechanisms by which colonic targeting may be achieved. Generally the mechanism of colon targeting can be grouped as follows.

### A. SPECIFIC

1. pH-sensitive polymers, which will dissolve at the pH associated with the cecal metabolism of polysaccharide (soluble fiber)

2. Azoreduction of polymers containing bonds that can be cleaved by reductive scission

3. Fermentable biopolymers in which the glycosidic bonds are broken by simple cleavage or more complete breakdown to short-chain fatty acids

### B. NONSPECIFIC

The other group of trigger mechanisms are fairly nonspecific, at least in terms of relying on the bacteria triggers, and may avoid premature release in the upper gastrointestinal tract by:

1. A combination of enteric coating and conventional time-dependent barrier coat dissolution.
2. Swelling systems that may eject or burst.
3. Eroding systems.
4. Those using slowed transit in the colon (pellet dosage forms) to release the majority of the drug when trapped in the ascending colon.

Every approach has pros and cons over each other and is more or less affected by the changes in diet, environmental conditions and diseased state. Colonic bacteria aided delivery systems are considered to be preferable and promising since the abrupt increase of the bacterial population and associated enzymatic activities in ascending colon represents a non-continuous event independent of GI transit time and pH. The critical component in bacteria aided systems is a series of polysaccharides such as xanthan gum, amylose, dextran, starch, chitosan, chondroitin, pectin, galactomannan which evade enzymatic degradation in the small intestine and are predominantly metabolized by colonic bacteria.[14]

The primary focus of this article is to review colon-specific delivery systems based on bacteria aided degradation of biopolymers. A brief description of physiological parameters of the colon relevant to colonic drug release, microflora of colon and various biopolymers that have potential to be used as a carrier, in unmodified or modified form, alone or in combination with other polymers, for colon specific drug delivery system, is also discussed.

**PHYSIOLOGICAL PARAMETERS AND COLONIC ABSORPTION**

In GIT large intestine starts from the ileo-caecal junction to the anus having a length of about 1.5 m (adults) and is divided into three parts, viz. colon, rectum and anal canal. The colon consists of caecum, ascending colon, transverse colon, descending colon and sigmoid colon. The successful targeted delivery of drug to colon via gastrointestinal tract (GIT) requires protection of drug from degradation and premature release in stomach and small intestine and then ensures abrupt or controlled release in the proximal colon. This necessitates a triggering element in the system that can respond to physiological changes in the colon. Overall, the physiological changes along the GI tract can be generally characterized as a continuum, with decreases in enzymatic activity, motility, and fluid content and an increase in pH. In normal healthy subjects, there is a progressive increase in luminal pH from the duodenum (pH = 6.6 + 0.5) to the terminal ileum (pH = 7.5 + 0.4), therefore, a decrease in the caecum (pH = 6.4 + 0.4), and then a slow rise from the right to the left colon with a final value of 7.0 ± 0.7.[15] These gradual changes in physiological parameters are not suitable for triggering elements to effect a sudden and dramatic change in the performance of a delivery system in order to obtain colon-specific delivery. However, the presence of specific bacterial populations in the colon and an apparent transient, small reversal in the otherwise increasing pH gradient are the exceptions that have been extensively explored as triggering components for initiating colon-specific drug release. Formulation of drugs for colonic delivery also requires careful consideration of drug absorption from colon, dissolution and/or release rate in the colonic fluids. The mucosa of the large intestine exhibits a much smaller surface because of a much smaller number of folds and villi relative to the small intestine. As a result, the absorption conditions are less favourable. In addition, the consistency of the intestinal contents becomes increasingly solid as the material flows in the direction of the ascending, traversing, and descending colon, until the normal consistency of faeces is obtained. Clearly, the absorption of drugs will be greatly influenced by this consolidation and decreasing diffusion rate. Because the large intestine membranes have a much smaller surface area, many investigators have postulated porous or permeable areas in the colonic membrane, like Peyers’ patches to explain the surprisingly good absorption for some drugs. Peyers’ patches are defined and anatomically discernable lymphatic folliculi aggregates. In addition to absorption through such permeable areas, it cannot be entirely excluded that absorption of water-soluble drugs is also facilitated by the considerable colonic dehydration flux. Blood flow to the colon is less than to small intestine, and the proximal colon receives a more prolific supply than the more distal regions. The nature of the drainage from the colon determines the fate of a drug taken up from this region. The drainage from the upper colon appears distinct from that of the more distal regions of the large intestine, where the former is drained by both the hepatic portal veins and the lymphatics and the latter is drained predominantly by the lymphatics.[16] Introduction of drugs in to the lymphatic, on the other hand, has been considered a means of improving oral bioavailability, because drugs could potentially passage from the lymphatic in to the blood circulation before reaching to the liver.[17] Generally, the dissolution and release rate from colonic formulations is thought to be decreased in the colon, which is attributed to the fact that less fluid is present in the colon than in the small intestine.[18] The poor dissolution and release rate may in turn lead to lower systemic availability of drugs. These issues could be more problematic when...
the drug candidate is poorly water-soluble and/or require higher doses for therapy. Consequently, such drugs need to be delivered in a presolubilized form, or formulation should be targeted for proximal colon, which has more fluid than in the distal colon. In spite of physiological barriers of colon some drugs have excellent bioavailability from this region and in some instance higher than that seen in small intestine. The colon also offers the advantage of lower efflux transporter level and lower metabolic enzyme levels which improve the bioavailability of some drugs. Local delivery to the colonic mucosa remains a valuable therapeutic option. New therapies that target inflammatory mediators could improve the treatment of inflammatory bowel disease, and old and new anticancer molecules could, when delivered topically, prove to be beneficial adjuncts to the current systemic or surgical treatments.\cite{19} Colon may also provide favourable site for protein and peptide absorption due to significantly lower levels of proteolytic enzyme as compared to small intestine and longer transit time for drug absorption.\cite{20}

**MICROFLORA OF THE COLON AND THEIR METABOLIC ACTIVITY**

Practically no problems exist in developing saliva- or gastric-resistant dosage forms. They can be designed on the basis of the considerable pH gradients between saliva and gastric juice and between gastric and intestinal juice, respectively. However no such reliable and sufficient pH gradient exists between the small and large intestine, alternative suitable gradients must be found. The most promising gradient in this regard is the vast difference in the microflora (i.e., in the bacterial counts between the small and large intestine). This is due to a retardation of movement of the contents or substratum within the gastrointestinal tract as a consequence of the widening of the intestinal lumen at the transition from the ileum in the caecum and the subsequent ascending first colon segment. Also, peristalsis is continuously decreasing from the small intestine to the end of the large intestine. These facts and the bag shaped nature of the caecum make this site a favourite region for microbial settlement. The intestine is adapted to bi-directional host–flora exchange and harbours a diverse bacterial community that is separated from the internal milieu by only a single layer of epithelial cells. Resident bacteria outnumber human somatic and germ cells tenfold and represent a combined microbial genome well in excess of the human genome.\cite{21} The intestinal microflora in the caecum is highly active. Collectively, the flora has a metabolic activity equal to a virtual organ within an organ.\cite{22} Although bacteria predominate in gut, archaea and eukarya are also represented. Acid, bile and pancreatic secretions hinder the colonization of the stomach and proximal small intestine by most bacteria. However, bacterial density increases in the distal small intestine, and in the large intestine rises to an estimated $10^{11}$–$10^{12}$ bacteria per gram of colonic content, which contributes to 60% of faecal mass. In addition to variations in the composition of the flora along the axis of the gastrointestinal tract, surface- adherent and luminal microbial populations also differ,\cite{23} and the ratio of anaerobes to aerobes is lower at the mucosal surface than in the lumen. In colon and faeces, the number of anaerobic bacteria is very high.

![Figure 1](image_url) Bacteria density increases in the jejunum/ileum from the stomach and duodenum, and in the large intestine. [Adapted from: Ann M.O’Haral & Fergus Shanahan. The gut flora as a forgotten organ: Review. EMBO Reports 7(2006) 688-693]
Table 1 The most commonly found intestinal anaerobic and aerobic bacteria

<table>
<thead>
<tr>
<th>Aerobic genera</th>
<th>Anaerobic genera</th>
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</thead>
<tbody>
<tr>
<td>Escherichia</td>
<td>Bifidobacterium</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Clostridium</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Bacteriodes</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>Eubacterium</td>
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</tbody>
</table>

The human colon is a dynamic and ecologically diverse environment, containing over 400 distinct species of bacteria [24] consisting mainly of non-sporing anaerobic bacteria. The genera bacteroides, Bifidobacterium, Eubacterium, clostridium, peptococcus, peptostreptococcus, and ruminococcus are predominant in human beings, whereas aerobes (facultative anaerobes) such as Escherichia, Enterobacter, Enterococcus, Klebsiella, lactobacillus, proteus, etc are among the subdominant genera. There are in addition a host of minor components of the flora including veillonellae, bacilli, peptococci and peptostreptococci, clostridia, Strep faecalis, and coliform organisms other than Esch. Coli (e.g., Klebsiella spp, Proteus spp). A major metabolic function of colonic microflora is the fermentation of non-digestible dietary residue and endogenous mucus produced by the epithelia. Most bacteria inhabit in the proximal areas of the large intestine, where energy sources are greatest. Fermentation of carbohydrates is a major source of energy for the bacteria in colon. Non-digestible carbohydrates include large polysaccharides (resistant starches, cellulose, hemicellulose, pectin, and gums), some oligosaccharides that escape digestion, and unabsorbed sugars and alcohols. The metabolic endpoint is generation of short-chain fatty acid [25].

For this fermentation, the microflora produces a vast number of enzymes like
1. Reducing enzymes: Nitroreductase, Azoreductase, N-oxide reductase, sulfoxide reductase, Hydrogenase etc.
2. Hydrolytic enzymes: Esterases, Amidases, Glycosidas, Glucuronidase, sulfatase, Glucuronidase, xylosidase, arabinosidase, galactosidase etc. There is certainly room for innovative approaches to carry and release drugs in the colon based on the metabolic capabilities of the colonic microflora [26].

However, only two or three enzyme systems have been exploited in this area: Azoreductase and Glycosidases (including Glucuronidase) for development of colon-specific drug delivery systems. A summary of the most important metabolic reactions carried out by bacterial enzymes in colon enzymes is summarized in Table 1 [27].

Table 2 List of bacterial enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Microorganism</th>
<th>Metabolic Reaction catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroreductase</td>
<td>E. coli, Bacteroides</td>
<td>Reduction of aromatic and heterocyclic nitro compounds</td>
</tr>
<tr>
<td>Azoreductase</td>
<td>Clostridia, Lactobacilli, E. Coli</td>
<td>Reductive cleavage of azo compounds</td>
</tr>
<tr>
<td>N-Oxide reductase, sulfoxide reductase</td>
<td>E. coli</td>
<td>Reduce N-Oxides and sulfoxides</td>
</tr>
<tr>
<td>Hydrogenase</td>
<td>Clostridia, Lactobacilli</td>
<td>Reduce carbonyl groups and aliphatic double bonds</td>
</tr>
<tr>
<td>Esterases and Amidases</td>
<td>E. coli, P. vulgaris, B. subtilis, B. myoides</td>
<td>Cleavage of esters or Amidases of carboxylic acids</td>
</tr>
<tr>
<td>Glucosidase</td>
<td>Clostridia, Eubacteria</td>
<td>Cleavage of , β-glycosidase of alcohols and phenols</td>
</tr>
<tr>
<td>Glucuronidase</td>
<td>E. coli, A. aerogenes</td>
<td>Cleavage of , β-Glucuronidase of alcohols and phenols</td>
</tr>
<tr>
<td>Sulfatase</td>
<td>Eubacteria, Clostridia, Streptococci</td>
<td>Cleavage of O-sulphates and sulfamates</td>
</tr>
</tbody>
</table>
The caecal bacteria in the right side of the colon largely control the characteristics of the lumen. Complex carbohydrates are fermented by the bacteria to small-chain fatty acids and carbon dioxide, the gas travelling to the transverse colon and being expelled through the lungs. The average bacterial load of the colon has been estimated at just over 200 g (equivalent to approximately 35 g dry weight). Water available for dissolution is maximal in the ascending colon and 1.5–2 L enters from the terminal small intestine each day. The amount of water present varies, being maximal in the period 4–8 h after ingestion of a meal. In the morning, the colon is often empty, and any material remaining in the ascending colon is slowly cleared. In the upright position, the gas produced by fermentation travels to the transverse colon and may limit access of the contents to water. It would be expected that the low water–high gas environment of the transverse colon limits dissolution of materials. It also limits ingress of water into impermeable devices. In the descending colon, devices become impacted into the 300 g of faecal contents. The surrounding material limits diffusion and provides a nonabsorbing reservoir. Therefore, unless the contents are cleared, there will be no absorption in this region. Thus targeted delivery to the large bowel should be directed toward the proximal colon. The ascending colon provides some water for dissolution. In addition, contents at the base of the colon will be stirred by the arrival of additional fluids from the gut as meals and accompanying secretions. This area also provides two cues that can be used for targeting: the change in pH and the unique nonmammalian metabolic profile provided by cecal bacteria.

**Bacteria aided colon specific drug delivery system**

During the past decade, a large number of delivery systems were developed with an intention of colon-targeted drug delivery. However, the majority was based on pH- and time-dependent concepts with limited in vivo evaluation. As the similarity in pH between the small intestine and the colon makes pH-dependent systems less reliable. For time-dependent formulations, the location of initial drug release predominantly depends on the transit time of the system in the GI tract. Despite the relative consistency of transit times in small intestine[28], the retention times in the stomach are highly variable that will result in a spread of initial release sites in the distal GI tract from time-dependent systems. Accelerated transit through different regions of the colon has been observed in the patients with the irritable bowel syndrome[29], the carcinoid syndrome and diarrhoea[29], and the ulcerative colitis.[30] Therefore, time-dependent systems are not ideal to deliver drugs colon-specifically for the treatment of colon-related diseases including ulcerative colitis. Furthermore, when designing systems for the treatment of such diseases, it will be desirable that the drug is released in a bolus fashion upon entry into the colon. [31] Colon microflora has gained significance as a preferable triggering component in the design of colon-specific drug delivery systems since the abrupt increase of the bacteria population and corresponding enzyme activities in the colon represent a non-continuous event independent of GI transit time. A large number of polysaccharides are actively hydrolyzed by colonic bacteria leading to the possibility of using naturally occurring biopolymers as drug carriers. In addition, Ethereal sulfate prodrugs or carboxylated prodrugs may be metabolized in the colon to the parent drug leading to local delivery in the colon. Azoreductases produced by colonic bacteria play a central role in a number of delivery systems, most notably in catalyzing the release of drug from a variety of low molecular weight polymeric prodrugs, polymeric coatings and crosslinked hydrogels. The reduction of azo-bonds is an oxygen sensitive reaction apparently mediated by low-molecular weight electron carriers with \( E_0 \) = -200 to -350 mV.[32] The bacterial group responsible for beta-glycosidase activity in colon are lactobacilli, bacteroides and Bifidobacterium. Colonic glycosidic-bond degrading enzymes are involved in the local metabolism of natural laxatives into their active moieties. A number of natural plant glycosides have been found to be delivered selectively to the colon, where they are metabolized to the aglycone and the sugar moiety.[32] The cathartic agents cascara sagrada and senna are both mixtures of glycosides. On hydrolysis by colonic bacteria, they yield biologically active aglycones which have cathartic activity. If the aglycone is administered orally, there is little or no cathartic activity, indicating that it may be inactivated in the upper GIT or absorbed before exerting its effect locally on the colonic mucosa.[33] Another recent approach to colonic drug delivery is based on the ability of colonic bacteria to depolymerise certain polysaccharides. It is well known that many plants and animal polysaccharides are not absorbed from the GIT and that humans do not produce enzymes capable of degrading these polysaccharides. Pectin, guar gum, alginic acid chondroitin, dextran and carrageenan are all examples of polysaccharides that are capable of passing intact through the upper GIT. These materials are degraded by gut bacteria and, hence,
can potentially be used to carry drugs to the large intestine; release of drugs occurs as these polymers are hydrolyzed.\textsuperscript{[34]} These polymers shield the drug from the environments of stomach and small intestine, and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by micro-organism, or degradation by enzyme or break down of the polymer back bone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength and release of the drug entity.\textsuperscript{[35]}

- Colon specific drug delivery based on activation by colonic microflora

A. Drug-saccharide conjugates

The unique luminal metabolic activity of colon makes it possible to direct drug-saccharide conjugates for topical treatment of inflammation process confined to its epithelium. Release of active moiety from the prodrug depends on specific enzyme or a specific membrane transporter. The enzymes like galactosidase, xylosidase, glycosidase and deaminase are mainly targeted for colonic drug delivery. The Drug-Saccharide conjugates are successful as colon drug carriers if they are hydrophilic and bulky as it minimizes their absorption from the upper GIT. Once it reaches to the colon, it is converted into a more lipophilic drug molecule that is then available for absorption. However prodrug will be considered as new chemical entity from regulatory aspect. So far this approach has been limited to drugs related to the treatment of Irritating Bowel Distress (IBD).

i) Glycosidic conjugates

The drugs can be conjugated with different sugar moieties to form glycosidase linkage which due to their bulky and hydrophilic nature cannot penetrate the biological membranes upon ingestion and are poorly absorbed from small intestine, but once they reach colon, they can be effectively liberated by bacterial glycosidases to release the free drug and facilitates the absorption by the colonic mucosa. Free steroids when administered orally are almost absorbed in small intestine and less than 1% of the oral dose reaches colon. Glycosidic prodrug of dexamethasone (dexamethasone-2-glucoside) and Prednisolone (prednisolone-2-glucoside) were prepared and evaluated for delivery of the steroids to the colon.\textsuperscript{[36]} The narcotic prodrug naloxone-β-d-glucoside also passes the small intestine unabsorbed and is enzymatically biodegraded after reaching the caecum.\textsuperscript{[37]}

ii) Glucuronide conjugates

Similar to glycoside conjugates are the glucuronide conjugates containing corticosteroids\textsuperscript{[38, 39]} They also show improvements in the therapeutic effect and in the reduction of side effects.

iii) Dextran conjugates

These are synthesized by direct attachment of a drug with a carboxylic group to dextran. They remain unchanged and unabsorbed in the gastrointestinal tract until they reach the caecum. Once there, dextranases of the colonic microflora cleave the ester bond, converting the prodrug to the effective drug. A series of drugs of various pharmacological classes were linked with dextran and modified dextrans and tested in animals. The results indicate that the breakdown of the conjugated prodrugs is mainly mediated by the colonic microflora. An instructive example is the prodrug naproxen-dextran.\textsuperscript{[40]} Budesonide-Dextran conjugates were synthesized as prodrug of Budesonide for oral controlled delivery of the major part of the drug to the colon without needing to coat the pellets. In vivo-efficacy was evaluated against acetic acid induced colitis in rat. Data indicated that Budesonide-dextran conjugate is effective in improving signs of inflammation in experimental model of colitis through selective delivery of the drug to the inflamed area.\textsuperscript{[41]}

B. Biopolymers as matrices or coating

These biodegradable swelling polymers are normally of natural origin and are degraded by the colonic microflora. These materials are fermentable oligosaccharides or polysaccharides. The metabolism of plant polysaccharides by microflora of the large intestine, and especially the fermentation of non-starch polysaccharides, have been extensively investigated.\textsuperscript{[42, 43]} The colonic microflora secretes a number of enzymes that are capable of hydrolytic cleavage of glycosidic bonds. These include β-d-glucosidase, β-d-galactosidase, amylase, pectinase, xylanase, α-d-xylosidase, and dextranases. The polysaccharide which is polymer of monosaccharide retains their integrity, because they are resistant to digestive action of GI enzymes, matrices of polysaccharides are assessed to remain intact in physiological environment of stomach and small intestine, as they reach colon they are acted upon bacterial polysaccharidases and results in degradation of the matrices. The ability of natural polymers i.e. the polysaccharides, from algal origin (e.g. Alginates), plant origin (e.g. pectin, guar gum, locust bean gum, khaya gum, konjac glucomannan) microbial origin (e.g. dextran, cyclodextrins,
xanthan gum) and animal origin (Chitosan, Chondroitin, Hyaluronic acid) to act as substrates for the bacterial inhabitants of the colon together with their properties, such as swelling. Film forming has appeal to area of colon specific drug delivery as it comprised of polymer with large number of derivatizable groups, with wide range of molecular weight, varying chemical composition, biocompatibility, low toxicity and biodegradability, yet a high stability.

The biodegradable polymers are hydrophilic in nature and may have limited swelling characteristics in acidic pH. However, these polymers swell in the more neutral pH of the colon. Although the rate of drug release is governed to a limited extent by physical factors such as diffusion and drug solubility, the major mechanism of drug release is by matrix erosion produced by enzymatic or microbial interaction with the polymers. To make polysaccharides less hydrophilic, hemi-synthesis operations (acetylation and methylation) have been conducted to create macromolecules. These macromolecules can be crosslinked to reduce the release kinetics of the drug entrapped.

The biodegradable polymers can be employed (a) in the formulation matrix, or (b) as a coat, alone or in combination. Many of these polymers have limited release control properties owing to high water solubility. Hence, they are employed in formulations in the following ways: (a) combination with synthetic non biodegradable polymers, or (b) synthetic modification such that solubility is decreased without compromising on their specific degradation in the human colon. The modification is normally done by introduction of groups by: (a) covalent linkages or (b) reversible complexation processes. The various biopolymers that have been studied for colon specific drug delivery are as follows:

a. Starch polysaccharides
   i) STARCH

Starch is a polymer, which occurs widely in plants. In general, the linear polymer, amylose, makes up about 20wt % of the granule, and the branched polymer, amylpectin, the remainder. The α-1, 4-link in both components of starch is attacked by amylases and the α-1, 6-link in amylopectin is attacked by glucosidase. The hydrophilic nature of the starch due to abundance of hydroxyl groups, is a major constraint that seriously limits the development of starch-based material for industrial applications. Chemical modification has been studied as a way to solve this problem and to produce water-resistant material. It may be hydroxypropylated, acetylated, carboxymethylated, or succinylate. Starch has been evaluated for colon-targeted delivery as enteric-coated capsules by Viliviam V.D. et al. [44]. The use of resistant starch was studied for the improvement of gut microflora and to improve clinical conditions related to inflammatory bowel diseases, immunostimulating activities, and protection from colon cancer. The administration of probiotic bacteria with optionally modified resistant starch as a carrier and growth medium to alter the gastrointestinal tract microbial populations has resulted in a number of significant advantages, such as protection, of probiotics, and as a carrier to deliver economically and efficiently to specific sites. The enhancement of a resident microorganism population in a selected site of the GIT and suppression of an undesirable microbial pathogen are the other claims made by the usage of these formulations containing modified resistant starch and a probiotic. Other applications include reducing the incidence of colorectal cancers or colonic atrophy. [45] Adriano V. Reis et al synthesized and characterized starch modified hydrogel which showed potential to be used as carrier for colon specific drug delivery. The hydrogel was prepared by cross linking polymerization of modified starch using sodium persulfate as initiating agent. [46] Mahkm et al. modified Chitosan crosslinked starch polymers for oral insulin delivery. Increasing the Chitosan content in the copolymer enhanced the hydrolysis in the SIF and thus led to slower release in intestinal pH. [47]

   ii) AMYLOSE

Fig 2 : Chemical structure of starch

Fig 3 : Chemical structure of amylose
Amylose is a linear polymer of glucopyranose units (α-1,4 d-glucose) linked through α-d-(1,4)-linkages. The molecule usually consists of around 1000–5000 glucose units. Amylose is resistant to pancreatic amylases but is susceptible to those of bacterial origin. It possesses the ability to form films which are water swellable and are potentially resistant to pancreatic-amylase (Leloup et al., 1991) but are degraded by colonic bacterial enzymes. Alone, the biopolymer becomes porous on hydration. Addition of ethyl cellulose produces a polymer mixture suitable for colon. Organic solvent based amylose–ethylcellulose films have also been evaluated as potential coatings for colonic drug delivery. These films were found to be susceptible to digestion by bacterial enzymes in a simulated colonic environment. The extent of digestion was directly proportional to the amylose content in the film. Amylose–ethylcellulose films were also evaluated by Siew et al for delivery of 5-ASA pellets to the colon. Drug release from the coated pellets was assessed under gastric and small intestinal conditions in the presence and absence of pepsin and pancreatin using dissolution methodology, and also within a simulated colonic environment involving fermentation testing with human faeces in the form of a slurry. Wilson et al explored the utility of the coating for colonic targeting of single unit tablet systems of 5-ASA. Drug release from the coated tablets was assessed under pH dissolution conditions resembling the stomach and small intestine, and also in conditions simulating the colon using a batch culture fermenter inoculated with human faecal bacteria. Drug release from the coated products was assessed under pH dissolution conditions resembling the stomach and small intestine, and also in conditions simulating the colon using a batch culture fermenter inoculated with human faecal bacteria. Results of various studies based on amylose–ethylcellulose films indicate that the colon-specificity can therefore be achieved using such systems by judicious choice of the appropriate ratio of amylose to ethylcellulose and coat thickness.

b. POLYSACCHARIDES FROM BACTERIAL SOURCE

i) Dextran

Dextran is a polysaccharide consisting of α-1,6 d-glucose and α-1,3 d-glucose units. Dextran hydrogels are stable when incubated at 37°C with the small-intestinal enzymes amyloglucosidase, invertase, and pancreatin. However, they are degraded by dextranases, which is a microbial enzyme found in the colon. Hovgaard and Brondsted prepared dextran hydrogels by cross-linking with disocyanate. These hydrogels were characterized by equilibrium degree of swelling and mechanical strength. The dextran hydrogels were degraded in vitro by enzyme dextranase and in-vivo in rats and human colonic fermentation. Release of entrapped hydrocortisone was found to depend on the presence of dextranases in the release medium. In absence of dextranase drug release was observed to be based on simple diffusion process. Thus it follows that dextran hydrogels are dextranase sensitive and may hold promise as carrier for colon specific drug delivery.

ii) Cyclodextrins

Cyclodextrins are cyclic oligosaccharide that consists of 6-8 glucose units. The cyclodextrins consist of six, seven, or eight glucose monomers arranged in a doughnut-shaped ring, which are denoted α, β, or γ cyclodextrin, respectively. The specific coupling of the glucose monomers gives the cyclodextrin a rigid, truncated conical molecular structure with a hollow interior of a specific volume. They are known to be barely capable of being hydrolyzed and only slightly absorbed in the passage through the stomach and small intestine, and are fermented by colonic microflora into small saccharides. An anti-inflammatory drug biphenyl acetic acid (BPAA) as a model drug was selectively conjugated onto one of the primary hydroxyl groups of alpha, beta and gamma cyclodextrins through an ester or amide linkage and was studied for in vivo drug release behaviour in rat GI tract by Kunihiro Minami et al. The investigation revealed that cyclodextrin prodrugs were stable in rat stomach and
small intestine and negligibly absorbed from these tracts. Three to six hours after oral administration most of the prodrug had moved to colon and caecum releasing BPAA which appeared in blood after 3 – 6 hrs. An anti-inflammatory drug 5-ASA was conjugated onto the hydroxyl groups of α-, β- and γ-cyclodextrins (CyDs) through an ester linkage, and the in vivo drug release behaviour of these prodrugs in rat’s gastrointestinal tract after the oral administration was investigated by Mei-Juan Zou et al.\textsuperscript{56} The study concluded that the 5-ASA concentration in the rat’s stomach and small intestine after the oral administration of CyD-5-ASA conjugate was much lower than that after the oral administration of 5-ASA alone. The lower concentration was attributable to the passage of the conjugate through the stomach and small intestine without significant degradation or absorption, followed by the degradation of the conjugate site-specific in the caecum and colon. The oral administration of CyD-5-ASA resulted in lower plasma and urine concentration of 5-ASA than that of 5-ASA alone.

iii) Xanthan gum

Xanthan gum is high molecular weight extracellular polysaccharide secreted by the micro-organism \textit{Xanthomonas campestris}. Xanthan gum is soluble in cold water and solutions exhibit highly pseudoplastic flow. Its viscosity has excellent stability over a wide pH and temperature range and the polysaccharide is resistant to enzymatic degradation. Xanthan gum exhibits a synergistic interaction with the galactomannan guar gum and locust bean gum (LBG) and the glucomannan konjac mannan. This results in enhanced viscosity with guar gum and at low concentrations with LBG. At higher concentrations soft, elastic, thermally reversible gels are formed with locust bean gum and konjac mannan.\textsuperscript{57} Thiruganesh Ramasamy and colleagues described colon targeted drug delivery systems for Aceclofenac using xanthan gum as a carrier.\textsuperscript{58} In this study, multilayer coated system that is resistant to gastric and small intestinal conditions but can be easily degraded by colonic bacterial enzymes was designed to achieve effective colon delivery of Aceclofenac. The Eudragit coated system exhibited gastric and small intestinal resistance to the release of Aceclofenac. The rapid increase in release of Aceclofenac in simulated colonic fluid was revealed as due to the degradation of the xanthan gum membrane by bacterial enzymes.
c. Plant Polysaccharides

i) PECTIN

![Chemical structure of pectin](image)

Fig 7: Chemical structure of pectin

Pectin is a polysaccharide found in the cell walls of plants containing α-1,4 D-galactouronic acid and 1,2 D-Rhamnose with D-galactose & D-arabinose side chains. Pectin is polymolecular and polydisperse. It contains a few hundreds to about 1,000 saccharide units in a chain-like configuration, corresponding to an average molecular weight between 50,000 and 150,000 Dalton. Pectin is completely fermented in colon by microflora with low esterified pectin being fermented faster than high esterified pectin. It appears that only a partial degradation is possible at the pH 2 to 4 conditions of stomach via side chain hydrolysis and at pH 5 to 6 conditions of small intestine via β-elimination of main chain or de-esterification. Amol Pharia et al formulated and evaluated Eudragit (S-100)-coated pectin microspheres for colon targeting of 5-fluorouracil (FU). Eudragit was used as protective coating on the microspheres makes them able to release the drug at the particular pH of colonic fluid. A combined mechanism of release was proposed, which combines specific biodegradability of polymer and pH-dependent drug release from the coated microspheres. The experimental results demonstrated that Eudragit-coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system. Nonetheless, pectin is an aqueous soluble polymer. The matrix made of pectin is prone to swelling as well as erosion in aqueous medium leading to premature drug release at upper gastrointestinal tract and thereby defeating its ability as colon-specific drug delivery vehicle. This drawback can however be adjusted by changing its degree of methoxylolation or by preparing calcium pectinate. Usually low methoxy pectins (LM pectin) that have more free carboxylic group can be cross-linked with divalent cations (e.g., calcium, zinc) to produce a more water insoluble pectinate gel which has the potential to be an effective vehicle for drug delivery. Pectins with low degree of methoxylolation can also undergo amidation of carboxylic acid groups. Furthermore, amidated pectins are more prone to form a rigid gel structure with divalent cations than non amidated pectin. Therefore, amidated LM pectins allow the formation of a more compact Ca- or Zn-pectinate network than nonamidated and/or high methoxy (HM) pectins. Munjeri and colleagues reported entrapment of Indomethacin and sulphamethoxazole inside amidated pectin, gelled in the presence of calcium. The drug-containing core was coated by a Chitosan polyelectrolyte complex to obtain the desired release pattern in simulated intestinal media. Calcium pectinate, the insoluble salt of pectin was used for colon targeted drug delivery of Indomethacin by Rubenstein et al. Sriamornsak and colleagues coated Theophylline pellets with calcium-pectinate and reported a pH-dependent in vitro release in 4 h. A review by Tin Wui Wong and colleagues on Pectin Matrix as Oral Drug Delivery Vehicle for Colon Cancer Treatment suggests that multi-particulate calcium pectinate matrix is an ideal carrier to orally deliver drugs for site-specific treatment of colon cancer as (a) cross linking of pectin by calcium ions in a matrix negate drug release in upper gastrointestinal tract, (b) multi-particulate carrier has a slower transit and a higher contact time for drug action in colon than single-unit dosage form. Surajit Das et al developed a delayed release formulation of resveratrol as multiparticulate pectinate beads by varying different formulation parameters. The effects of the formulation parameters were investigated on shape, size, Zn content, moisture content, drug encapsulation efficiency, swelling–erosion, and resveratrol retention pattern of the formulated beads. The results indicated that Zinc-pectinate beads exhibited better delayed drug release pattern than calcium-pectinate beads.

ii) Guar gum

![Chemical structure of guar gum](image)

Fig 8: Chemical structure of guar gum
Guar gum is a galactomannan polysaccharide ($\beta$-1,4 d-mannose, $\alpha$-1,6 d-galactose) having ($1\rightarrow 4$) linkages. It has a side-branching unit of monomeric d-galactopyranose joined at alternate mannose unit by ($1\rightarrow 6$) linkage. It has low water solubility but hydrates and swells in cold water forming viscous colloidal dispersions or sols. The viscosity of a guar gum solution incubated with a homogenate of faeces will be reduced by 75% over 40 min. \[67\] It is susceptible to galactomannase enzyme in the large intestine. Wong and colleagues reported the evaluation of the dissolution of dexamethasone and Budesonide from guar-gum-based matrix tablets. \[68\]

The presence of low-grade HPMC (Methocel E3) or higher-grade HPMC (Methocel E50 LV) in dexamethasone formulations altered the rate of the matrix degradation. \[69\] Krishnaiah et al. \[70\] described a Scintigraphic study using technetium-99m-DTPA as a tracer, incorporated into tablets to follow the transit and dissolution. Scintigraphic scans revealed that some tracer was released in stomach and small intestine but the bulk of the tracer present in the tablet mass was delivered to the colon. Results of pharmacokinetic studies of guar gum based colon targeted drug delivery system of mebendazole and 5-Flourouracil in healthy volunteers indicated that guar gum based colon targeted tablets of did not release drug in stomach and small intestine but delivered the drug in colon resulting in slow absorption of the drug and making the drug available for local action in the colon. \[71\]

Rubinstein and Gliko-Kabir \[72\] reported cross-linking of guar gum with borax to enhance its drug-retaining capacity. Guar gum as a film coating material for colon specific drug delivery of 5-Fluorouracil was evaluated by C.M.Ji and colleagues. \[73\] The guar gum based multi unit system was prepared by coating guar gum and pH sensitive polymer Eudragit FS30D around drug loaded cores. Eudragit was used to protect the system against gastrointestinal environment having pH less than 7. The in vitro results indicated that guar gum is a feasible coating material to achieve time and enzyme triggered 5-Flourouracil release. Laila Fatimaali Asghar et al \[74\] assessed the suitability of guar gum with pH sensitive polymer matrix baxes for colon specific delivery using Indomethacin as model drug and EL 100 and ES 100 as Ph sensitive polymers. The study concludes that mixed polymer matrix with pH modulated properties can serve as an alternative to coating technology which although has commercial feasibility, yet suffers from the drawback of inconsistent performance in vivo. A pH and time controlled matrix system can offer a suitable platform for colon targeting purpose with minimum drug loss during upper GI transit and maximum drug release in the colon. Mohini Chaurasia et al \[75\] has evaluated crosslinked guar gum microspheres for improved delivery of anticancer drug methotrexate in the colon for treatment of colorectal cancer. Results of release studies demonstrated that microspheres are capable of retarding the release of MTX until it reaches the colon, an environment rich in bacterial enzymes that degrade the guar gum and allow drug release to occur at the desired site.

iii) Inulin

Inulin is a naturally occurring Glucofructan and occurs in plants such as garlic, onion, artichoke and chicory. It can resist hydrolysis and digestion in the upper gastrointestinal tract but is degraded by colonic microflora. Inulin, with a high degree of polymerization, was formulated as a biodegradable colon-specific coating by suspending it in Eudragit RS films. The films withstood gastric and intestinal fluid but were degraded by faecal media. \[76\] Vinyl groups were introduced in inulin chains to form hydrogels, by reacting with glycidyl methacrylate. \[77\] Enzymatic digestibility of the prepared hydrogels was assessed by performing an in vitro study using an inulinase preparation derived from Aspergillus niger. Equilibrium swelling ratio and mechanical strength of the hydrogels were also studied. Based upon the mode of swelling it was concluded that inulin-degrading enzymes were able to diffuse into the inulin hydrogel networks causing bulk degradation. \[78\] Inulin derivatised with methacrylic anhydride and succinic anhydride produced a pH sensitive hydrogel by UV irradiation that exhibited a reduced swelling and low chemical degradation in acidic medium, but it had a good swelling and degradation in simulated intestinal fluid in the presence of its specific enzyme, inulinase. \[79\]

iv) GUM KARAYA

Gum karaya is a complex, partially acetylated polysaccharide obtained as a calcium and
magnesium salt. It has a branched structure and a high molecular mass of approximately $16 \times 10^6$ Da.\[80\] The backbone of the gum consists of α-D-galactouronic acid and α-L-Rhamnose residues. Side chains are attached by 1, 2-linkage of β-D-galactose or by 1,3-linkage of β-D glucuronic acid to the galactouronic acid of the main chain. Furthermore, half of the Rhamnose residues of the main chain are 1,4-linked to β-D-galactose units.\[81\] The chemical composition of gum samples obtained from different Sterculia species and from different places of origin was found to be quite similar.\[82\] The solubility of gum karaya in water is poor. However, the gum swells up to many times its own weight to give dispersions.\[83\] Baljit B Singh et al modified sterculia gum with methacrylic acid to form hydrogels which were evaluated for release mechanism using ranitidine hydrochloride as model drug.\[84,85\] Jitendra R. Amrutkar and colleague has described study of a novel hydrogel plug prepared using isolated root mucilage of S. urens for colon specific pulsatile drug delivery of Indomethacin. Pulsatile drug delivery was developed using chemically treated hard gelatin capsule bodies filled with Eudragit multiparticulates of Indomethacin, and sealed with different hydrogel plugs (root mucilage of S. urens, xanthan gum, guar gum, HPMC K4M and combination of maltodextrin with guar gum). The formulation factors affecting the drug release were concentration and types of hydrogel plug used. In vivo gamma Scintigraphic study in healthy rabbits proved the capability of the system to release drug in lower parts of the gastrointestinal tract after a programmed lag time.\[86\] The results suggest that gum karaya has potential for drug targeting to the colon. But till date not many drug delivery systems have been investigated utilizing this gum for targeting to colon.

v) Locust bean gum

Locust bean gum (chemical structure is shown in Figure 8) also known as Carob bean gum is derived from the seeds of the leguminous plant Ceratonia siliqua Linn. This gum is widely cultivated in the Mediterranean region and to a smaller extent also in California. The brown pods or beans of the locust bean tree are processed by milling the endosperms to form locust bean gum and it is therefore not an extract of the native plant but flour. Locust bean gum consists mainly of a neutral galactomannan polymer made up of 1, 4-linked D-mannopyranosyl units and every fourth or fifth chain unit is substituted on C-6 with a D-galactopyranosyl unit. The ratio of D-galactose to D-mannose differs and this is believed to be due to the varying origins of the gum materials and growth conditions of the plant during production. Locust bean gum is a neutral polymer and its viscosity and solubility are therefore little affected by pH changes within the range of 3-11.\[87\] Various significant works have been carried out in combination with the other polymers to make the formulation sustained and targeted. The colon specific drug delivery of mesalazine based on polysaccharides; locust bean gum and Chitosan in different ratios were evaluated using in vitro and in vivo methods by Raghavan CV et al.\[88\] The in vivo studies conducted in nine healthy male human volunteers for the various formulations revealed that, the drug release was initiated only after 5 h (i.e.) transit time of small intestine. These studies on the polysaccharides demonstrated that the combination of locust bean gum and Chitosan as a coating material proved capable of protecting the core tablet containing Mesalazine during the condition mimicking mouth to colon transit. In particular, the formulation containing locust bean gum and Chitosan in the ratio of 4 : 1 held a better dissolution profile, higher bioavailability and hence a potential carrier for drug targeting to colon.

vi) Konjac glucomannan (KGM)

Fig 10 : Chemical structure of locust bean gum

Fig 11 : Chemical structure of KGM
Konjac glucomannan (KGM), a water-soluble and high molecular weight polysaccharide, is extracted from tubers of the Amorphophallus Konjac plant, a member of family Araceae found in East Asia. It consists of 1,4-linked D-mannose and D-glucose in the ratio of 1.6:1, with about 1 in 19 units being acetylated. This soluble fibre has high water holding capacity, forming a highly viscous solution. In recent years, KGM gel, as a drug delivery matrix, has shown promising applications. Strong, elastic, heat-stable KGM gels can be formed with heating and mild alkali and can be used as a drug carrier.

Hydrogel systems of KGM cross-linked with trisodium trimetaphosphate were prepared for colon targeting drug delivery. KGM unmodified and in combination with xanthan gum has also been evaluated as carrier for colon targeting. Kang et al. have described experimental and theoretical evaluations of Konjac glucomannan and xanthan gum as compression coating for colon drug delivery of Cimetidine. Diffusion of Cimetidine from compression coated tablets was investigated by release experiment in vitro. 0.22U/mL β-mannanase was applied in the mimic colon solution. The experimental results indicate that the polysaccharide mixtures of KGM and XG as the compression coat have a great potential in the application of colonic drug delivery systems. The synergistic interaction between XG and KGM reduced drug loss in the mimic upper gastrointestinal solution. At the same time, the coat maintained a good response to degradation due to the hydrolysis of KGM. Alvarez-Manceñido F et al studied release of of a typically highly water soluble drug Diltiazem from sugar matrices tablets prepared using binary mixture of Konjac glucomannan and xanthan gum, in presence of A. niger beta mannose (used to mimic colonic enzyme). Drug release from these tablets remained zero-order, but was accelerated (presumably due to degradation of KGM), in the presence of A. niger beta-mannanase at concentrations equivalent to human colonic conditions. However, marked differences between Japanese and American varieties of KGM as regards degree of acetylation and particle size led to significant differences in swelling rate and drug release between formulations prepared with one and the other KGM: whereas a formulation with Japanese KGM released its entire drug load within 24h in the presence of beta-mannanase, only 60% release was achieved under the same conditions by the corresponding formulation with American KGM. The results of this study suggest that sustained release of water-soluble drugs in the colon from orally administered tablets may be achieved using simple, inexpensive formulations based on combinations of KGM and XG.

vii) Khaya gum
Khaya gum is a polysaccharide obtained from the incised trunk of the tree Khaya grandifoliola (family Meliaceae). It is known to contain highly branched polysaccharides consisting of D galactose, L-Rhamnose, D-galactouronic acid and 4-O-6 methyl-D-glucuronic acid. The colon specificity of Khaya gum was investigated in comparison with guar gum by Prabhakra Prabhu et al using Budesonide as drug core. The tablets were coated with Khaya gum or Guar gum followed by further coat or Eudragit L 100 for both. X-ray images were taken to investigate the movement, location and the integrity of the tablets in different parts of gastro intestinal tract in rabbits. Dissolution models employed revealed colon specificity of both the polysaccharides however, Khaya gum or Guar gum alone can not be used either for targeting the drug to the colon or for sustaining or controlling the release of drug. Khaya and Albizia gums were evaluated as compression coatings for target drug delivery to the colon using Indomethacin (a water insoluble drug) and paracetamol (a water soluble drug) as model drugs. The core tablets were compression-coated with 300 and 400mg of 100% khaya gum, 100% Albizia gum and a mixture of khaya and Albizia gum (1:1). Drug release studies were carried out in 0.1M HCl (pH 1.2) for 2h, Sorensen's buffer (pH 7.4) for 3 h and then in phosphate-buffered saline (pH 6.8) or in simulated colonic fluid for the rest of the experiment to mimic the physiological conditions from the mouth to colon. The results indicated that khaya and albizia gums were capable of protecting the core tablet in the physiological environment of the stomach and small intestine, with Albizia gum showing greater ability than khaya gum. The results demonstrate that khaya gum and Albizia gum have potential for drug targeting to the colon.

d. Polysaccharides of animal origin
   i) Chondroitin Sulfate

Fig 12 : Chemical structure of chondroitin
Chondroitin sulfate is a soluble mucopolysaccharide consisting of β-1,3 d-glucuronic acid linked to N-acetyl-d-galactosamine. In the human colon, the natural sources of Chondroitin sulphate are sloughed epithelial cells and dietary meat. Chondroitin sulphate is utilized as a substrate by the *Bacteroides thetaiotaomicron* and *B. ovatus* in the large intestine. Natural chondroitin sulfate is readily water soluble and may not be able to sustain the release of many drugs from the matrix. However modification of chondroitin sulphate by cross linking reduces its hydrophilicity and prevents early release of the drug. Rubinstein and co-workers have reported the use of cross-linked chondroitin sulfate as a carrier for Indomethacin specifically for the large bowel. Since natural chondroitin sulfate is readily water soluble, it was cross-linked with 1, 12-diaminododecane. The cross-linked polymer was blended with Indomethacin and compressed into tablets. There was enhanced release on incubation with rat cecal contents. Jitendra R. Amrutkar et al prepared polyelectrolyte complex of Chitosan and chondroitin and studied its potential as colon targeted carrier by preparing matrix tablet of Indomethacin. The study confirmed that selective delivery of Indomethacin to the colon can be achieved using cross-linked Chondroitin and Chitosan polysaccharides. The dissolution data indicated that the dissolution rate of the tablet is dependent upon the concentration of polysaccharide used as binder and matrix and time of cross-linking. R. Thiruganesh et al used a combined approach of Ph dependent polymeric coating (Eudragit L 100 and S 100) and microbially degradable Chitosan to develop a single unit site specific matrix tablet of Aceclofenac. The coating polymers were included for protecting the drug from releasing from the core before reaching the colonic region.

**ii) Chitosan**

Chitosan is a fiber-like substance derived from chitin. Chitin and Chitosan have similar chemical structures. Chitin is made up of a linear chain of acetyl glucosamine groups, while Chitosan is obtained by removing enough acetyl groups for the molecule to be soluble in most dilute acids. This process is called deactivation. The actual difference between chitin and Chitosan is the acetyl content of the polymer. Chitin is widely available from a variety of source, among which the principal one is shellfish waste such as shrimps, crabs, and crawfish. The degree of de acetylation of Chitosan ranges from 56% to 99% with an average of 80%, depending on the crustacean species and the preparation methods. Chitin with a degree of de acetylation of 75% or above is generally known as Chitosan. Chitosan is a non toxic, biodegradable polymer of high molecular weight, and is very much similar to cellulose, a plant fiber. The only difference between Chitosan and cellulose is the amine (–NH2) group. However, unlike plant fiber, Chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules. It is a poly (2-amino 2-deoxy d-glucopyranose) in which the repeating units are linked by (1–4) β-bonds. It dissolves in the acidic pH of the stomach but swells at pH 6.8. Chitosan can be biodegraded by colonic microflora and has been evaluated for its potential as colon-specific drug delivery in several forms such as capsules, matrices, hydrogels and microspheres. Tominga and colleagues prepared a composite for delivery to the colon comprising an active core, an internal layer comprising Chitosan, and an external layer, coated on the internal coating layer, containing zein. Zein protects the contents by being acid resistant but undergoing proteolysis in the small intestine. Chitosan in the internal layer prevents the elution of the active ingredients in the core in the small intestine. However, in the large intestine the Chitosan film breaks owing to the combined effect of microorganisms and osmotic pressure. Hideyuki Tozaki and colleagues conducted a study to estimate colon specific insulin delivery with Chitosan capsules. The intestinal absorption of insulin was evaluated by measuring plasma insulin level and its hypoglycaemic effect after oral administration of Chitosan capsules containing insulin. The hypoglycaemic effect started after 8 hrs of administration of Chitosan capsules. The findings of the study suggest that Chitosan capsules may be useful carriers for the colon specific delivery of peptides including insulin. The pH-sensitive multicore microparticulate system
containing Chitosan microcores entrapped into enteric acrylic microspheres has been reported by M.L. Lorenzo-Lamosa et al. Sodium diclofenac was efficiently entrapped within these Chitosan microcores and then microencapsulated into Eudragit L-100 and Eudragit S-100 to form a multi reservoir system. In vitro release study revealed no release of the drug in gastric pH for 3 h and after the lag-time, a continuous release for 8–12 h was observed in the basic pH. A microbially triggered colon-targeted osmotic pump (MTCT-OP) has been studied by Hui Liu et al. The gelable property of Chitosan at acid condition and colon-specific biodegradation of Chitosan were used to produce the osmotic pressure, formation of the drug suspension and formation of in situ delivery pores for colon-specific drug release, respectively. These results showed that MTCT-OP based on osmotic technology and microbially triggered mechanism had a high potential for colon-specific drug delivery.

iii) Hyaluronic Acid

Fig 14 Chemical structure of Hyaluronic acid (HA)

Hyaluronic acid (HA) is a carbohydrate, more specifically a mucopolysachharide, occurring naturally in all living organisms. It can be several thousands of sugars (carbohydrates) long. When not bound to other molecules, it binds to water giving it a stiff viscous quality similar to “Jello”. It consists of N-acetyl-D glucosamine and β-glucuronic acid. It is present in the intercellular matrix of most vertebrate connective tissues especially skin where it has a protective, structure stabilizing and shock-absorbing role. It is found in greatest concentrations in the vitreous humour of the eye and in the synovial fluid of articular joints. Commercially produced HA is isolated either from animal sources, within the synovial fluid, umbilical cord, skin, and rooster comb, or from bacteria through a process of fermentation or direct isolation. The molecular weight of HA is heavily dependent on its source; however, refinement of these isolation processes has resulted in the commercial availability of numerous molecular weight grades extending up to a maximum of 5000 k Da. The hydrogen bond formation results in the unique water-binding and retention capacity of the polymer. It also follows that the water binding capacity is directly related to the molecular weight of the molecule. Up to six litres of water may be bound per gram of HA. HA solutions are characteristically viscoelastic and pseudoplastic. This rheology is found even in very dilute solutions of the polymer where very viscous gels are formed. The viscoelastic property of HA solutions which is important in its use as a biomaterial is controlled by the concentration and molecular weight of the HA chains. The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications, including the supplementation of joint fluid in arthritis, as a surgical aid in eye surgery, and to facilitate the healing and regeneration of surgical wounds. More recently, HA has been investigated as a drug delivery agent for various administration routes, including ophthalmal, nasal, pulmonary, parenteral and topical. In mammals, the enzymatic degradation of HA results from the action of three types of enzymes: hyaluronidase (hyase), β-d-Glucuronidase, and β-N-acetyl-hexosaminidase. Throughout the body, these enzymes are found in various forms, intracellularly and in serum. It has been shown that the HA level is elevated in various cancer cells. The higher concentration of HA in cancer cells is believed to form a less dense matrix, thus enhancing the cell’s motility as well as invasive ability into other tissues and also providing an immunoprotective coat to cancer cells. It is well known that various tumors, for example, epithelial, ovarian, colon, stomach, and acute leukaemia, overexpress HA-binding receptors CD44 and RHAMM. Consequently, these tumor cells show enhanced binding and internalization of HA. It has been shown that the over expression of hyaluronic acid syntheses increases the HA level, which leads to the acceleration of tumor growth and metastasis. On the other hand, exogenous oligomeric HA inhibits tumor progression most likely by competing with endogenous polymeric HA. HA can be coupled with an active cytotoxic agent directly to form a non-toxic prodrug. Alternatively, a suitable polymer with covalently attached HA and drug can be used as a carrier. Direct conjugations of a low molecular weight HA to cytotoxic drugs such as butyric acid, paclitaxel, and doxorubicin have been reported. It has been shown that these bio conjugates
are internalized into cancer cells through receptor-mediated endocytosis, followed by intracellular release of active drugs, thus restoring their original cytotoxicity. Hyaluronic acid–coupled Chitosan nanoparticles bearing oxaliplatin (L-OHP) encapsulated in Eudragit S100–coated pellets were developed for effective delivery to colon tumors by Anekant Jain et al.\textsuperscript{[123]} The in vitro drug release was investigated using a USP dissolution rate test paddle-type apparatus in different simulated gastrointestinal tract fluids. In therapeutic experiments the pellets of free drug, and hyaluronic acid–coupled and uncoupled Chitosan nanoparticles bearing L-OHP were administered orally at the dose of 10 mg L-OHP/kg body weight to tumor-bearing Balb/c mice. In vivo data showed that hyaluronic acid–coupled Chitosan nanoparticles delivered 1.99 ± 0.82 and 9.36 ± 1.10 g of L-OHP/g of tissue in the colon and tumor, respectively after 12 hr. These drug delivery systems showed relatively high local drug concentration in the colonic milieu and colonic tumors with prolonged exposure time, which provides a potential to enhance antitumor efficacy with low systemic toxicity for the treatment of colon cancer and thus indicating its targeting potential to the colon and tumor. Although being used since many years this interesting biopolymer needs to be explored as carrier for microbially triggered oral colon specific drug delivery system.

CONCLUSION:
Targeting drugs and delivery systems to the colonic region of the gastrointestinal tract has received considerable interest in recent years and bacteria aided biopolymers appear to be promising agents for obtaining colon-specific drug delivery systems. A successful colon specific drug delivery requires that the system responds only to those physiological conditions prevailing in colon. A variety of delivery strategies and systems have been proposed for colonic targeting. These generally rely on the exploitation of one or more of the following gastrointestinal features for their functionality: pH, transit time, pressure or microflora. Coated systems that utilise the pH differential in the gastrointestinal tract and prodrugs that rely on colonic bacteria for release have been commercialised. Both approaches have their own inherent limitations. The use of natural biopolymers for colon specific drug delivery system remains attractive because of their abundance in nature, good biocompatibility, and ability to be readily modified by simple chemistry and most distinctive property of colon that is abundant microflora. The biopolymers described with polyvalent ions such as calcium or aluminium allow cross-linking with subsequent formation of gels that can be employed to prepare matrices, films, beads, pellets, microparticles and nanoparticles.\textsuperscript{[125]} Crosslinked alginates has more capacity to retain the entrapped drug and mixing of alginates with other polymers such as neutral gums, pectins, Chitosan and Eudragits have been found to solve the problems of drug leaching. The sustained release profiles of single and dual crosslinked gel beads of Alginate-Chitosan loaded with bovine serum albumin (BSA), a model protein drug, were investigated in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF). Alginate-Chitosan (ALG-CS) blend gel beads were prepared based on dual cross linking with various proportions of algin and Chitosan. The dual cross linkage effectively promoted the stability of beads under gastrointestinal tract conditions compared to Ca\textsuperscript{2+} single crosslinked beads, from which BSA released fast and the cumulative drug release percentages were about 80% of all formulations in 4 h, the BSA total release from dual crosslinked gel beads was no more than 3% in 8 h. In SIF and SCF, Ca\textsuperscript{2+} single crosslinked beads were disrupted soon associated with the fast drug release as compared to the dual crosslinked beads, which suggested that the dual crosslinked beads have potential small intestine or colon site-specific drug delivery property.\textsuperscript{[126]}

e. Polysaccharides Of Algal Origin
i) Alginates

![Fig 15: Chemical structure of alginate](image)

Alginates or alginic acids are linear unbranched polysaccharides found in brown seaweed and marine algae such as Laminaria hyperborea, Ascophyllum nodosum and Macrocystis pyrifera. These polymers consist of two different monomers in varying proportions, namely β-D-mannuronic acid and α-L-guluronic acid linked in α- or β-1,4 glycosidic bonds as blocks of only β-D-mannuronic acid or α-L-guluronic acid in homopolymers or alternating the two in heteropolymers blocks. Alginates have high molecular weights of 20 to 600 kDa.\textsuperscript{[124]} The gelling properties of alginate’s glucuronic residues
above and their degradation products are non toxic and are already used as pharmaceutical excipients. The colonic microflora does not appear to present many modifications and remains qualitatively similar from one individual to another. Chemical modifications like chemical linkages with synthetic biopolymers; surface coating of pellets, micro- or nanospheres with biocompatible synthetic polymers; Crosslinking with different physical or chemical reagents; Hydrophobization through alkylation reactions, made to biopolymers make it possible to decrease the water solubility of biopolymers and control the lag time of drug release. Needs for chemical modification concern mainly the improvement of mechanical properties, biocompatibility, solubility, control of biodegradability and manufacturing and shaping. Although offering an attractive tool, bacteria aided colonic delivery systems using biopolymers suffer from the constraint of premature release of their drug load to a certain extent in the upper segments of the GI tract. This early discharge is inherent and associated with the swelling of the carrier, a crucial process, which allows cleavage by colonic enzymes. For that reason, most enzymatically controlled colonic drug carriers cannot function optimally without the aid of a protective coat (primary carrier), whether pH-dependent or depending on the erosion of a physical barrier. Combinations of natural biopolymers with pH dependant synthetic polymers have been studied for colon specific drug delivery, which are based on erosion and swelling of film coating all along the gastrointestinal tract and microbial triggered degradation of polysaccharide. It is, however, necessary to obtain more knowledge of the bacterial flora and to develop and validate a dissolution method that incorporates the physiological features of the colon, particularly enzymatic degradation and yet can be used routinely in an industry setting for the evaluation of colon-specific drug delivery systems.

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