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Current Research in Pharmaceutical Sciences

Available online at www.crpsonline.com

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ISSN 2250 – 2688

Received: 02/09/2015
Revised: 21/09/2015
Accepted: 29/09/2015

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Polysaccharides for Bacterially Triggered System in Colon Targeting

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ABSTRACT

Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug to the colon. The rationale for the development of a polysaccharide based 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. Various major approaches utilizing polysaccharides for colon-specific delivery are fermentable coating of the drug core, embedding of the drug in biodegradable matrix, formulation of drug-saccharide conjugate (prodrugs). A large number of polysaccharides have already been studied for their potential as colon-specific drug carrier systems, such as chitosan, pectin, chondroitin sulphate, cyclodextrin, dextrans, guar gum, inulin, amylose and locust bean gum.. The colon specific delivery systems based on a single polysaccharide do not efficiently permit targeted release. The pH and transit time can vary depending on the individual and the particular disease state. The conventional approaches give rise to premature drug release. The combination/chemically modified forms of polysaccharides eliminated the drawbacks associated with the use of single polysaccharide. Recent efforts and approaches exploiting these polysaccharides in colon-specific drug delivery by bacterially triggered systems.

Keywords: Colon-specific drug delivery, Colon targeting, Polysaccharides, Carbohydratepolymer, Bacterially triggered system

1. INTRODUCTION

The oral route is considered to be most convenient for administration of drugs to patients. The conventional oral dosage forms normally dissolve in the stomach fluid or intestinal fluid and are absorbed from these regions of the Gastrointestinal Tract (GIT), which depend upon the physicochemical properties of the drug. Localized delivery of the drugs in the colon region is possible only when the drug is protected from the hostile environment of upper GIT. Dosage forms that deliver drugs into the colon region rather than upper GIT proffers number of advantages. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, Chron's disease, carcinomas and infections) whereby high local concentration can be achieved while minimizing side effects that occur because of release ofdrugs in the upper GIT or unnecessary systemic absorption. The colon is rich in lymphoid tissue.Uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery[1]. Specific systemic absorption of drugs and protein/peptides in the colonic region offers interesting possibilities for the treatment of disease susceptible to diurnal rhythm such as asthma, arthritis or inflammation[2,3]. The colon is considered to be more suitable for delivery of peptides and protein in comparison to small intestine [4,5]. Besides this low hostile environment, the colonic transit time (20-30 hours) and the colonic tissue is highly responsive to the action of absorption enhancers [6,7]. Colonic delivery can be accomplished by oral or rectal administration. Rectal dosage forms such as suppositories and enemas are notalways effective since a high variability in the distribution of these forms is observed [8-10].The GIT is divided into various regions like stomach, small intestine and large intestine.

The colon serves four major functions: viz; creation of suitable environment for the growth of colonic microorganisms, storage reservoir of faecal contents, expulsion of the contents of the colon at an appropriate time, absorption of potassium and water from lumen and excretion of potassium and bicarbonate.

The upper part of GIT, i.e. the stomach and the duodenum has a microflora of less than 10^3–10^4 CFU/ml. These are mainly gram-positive facultative bacteria. The microflora of colon on the other side is in the range of 10^{11}–10^{12} CFU/ml consisting mainly of anaerobic bacteria, e.g. *Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria,* etc. This vast microflora fulfils its energy needs by fermenting various types of substrates that have been left undigested in the small intestine, e.g, di- and tri-saccharides, polysaccharide etc. For this fermentation, the microflora produces a vast number of enzymes like β-glucuronidase, β-xylosidase, α-arabinosidase, β-galactosidase, nitroreductase, azoreductase, and deaminase and urea dehydroxylase. Because of the presence of these biodegradable enzymes only in the colon, the use of bacterial degradable polymers for colon-specific drug delivery seems to be a more site specific approach as compared to other approaches. These polymers shield the drug from the environments of the stomach and the 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 breakdown of the polymer backbone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer.

Oxygen is a limiting factor for the growth of colonic microflora the metabolic activity of which can be affected by factors such as age, gastrointestinal diseases, the administration of drugs, the fermentation of food residues. All of these factors can lead to the inactivation of drugs or to the development of certain side effects.The enzymes involved in the metabolism of the drugs or other compounds are, exclusively, certain types of reducing enzymes or enzymes involved in degradation by hydrolysis. The presence and capacities of such enzymes have been used to specifically cleave certain types of drugs attached to another molecule or a polymer and then to develop the concept of prodrugs or conjugated polymers. The drugs are generally attached by a nitrogenous double bond. Cleavage of the drugs is achieved in the anaerobic and reducing medium by the azo-reductase enzyme and certain biomolecules, such as NADPH. The same concept has been also used to synthesise polymers with nitrogenous double bonds or disulfide bonds in the backbone. Using these polymers as coatings for dosage forms, they must also be specifically disintegrated by the action of the enzymes and the microflora.

The various approaches used for targeting the drugs to the colon include, formation of a prodrug, multicoating time-dependent delivery systems, coating with pH-sensitive polymers, pressure dependent systems, and the use of biodegradable polymers.

A prodrug is a pharmacologically inactive derivative of a parent molecule that requires spontaneous or enzymatic transformation within the body to release the active drug moiety. For targeting drugs to the colon, drug is to be protected from the hostile environments of the stomach and small intestine (SI). This protection in the upper GIT is affected by conjugation with carrier moieties, forming prodrugs. These prodrugs undergo enzymatic cleavage in the colon and regenerate the drug. An example of such a prodrug, which is extensively used in Crohn's disease and ulcerative colitis is sulphasalazine[11]. It consists of 5-aminosalicylic acid (5-ASA) linked via an azo bond to sulphapyridine (SP). This prodrug when given orally is minimally absorbed in the stomach and the small-intestine and largely reaches the colon, where the bacterial azoreductase cleaves the azo bond thereby releasing 5-ASA, the drug moiety from SP, which acts only as a carrier[12]. Glycosidic prodrugs,[13-15] dextran prodrugs[16] and cyclodextrin conjugated prodrugs [17] of various drugs have been developed for colon-specific drug delivery.

Time dependent formulations are designed to resist the release of the drug in the stomach with an additional non-disintegration or lag phase included in the formulation (which equals to the small intestinal transit time) and the release of the drug takes place in the colon. An example of such a system is Pulsincap. This capsule consists of a non-disintegrating body having an enteric coated cap. The enteric coated cap dissolves in the small intestine and a hydrogel plug swells to create a lag phase. This plug ejects on swelling and releases the drug from the capsule. The large scale manufacturing of these systems, however, needs a lot of technological advancement and skills. Another limitation of the time dependent release systems are the variation in the gastric emptying time and small intestinal transit time[18]. But, due to the use of enteric coating over most of these systems, the large variation in gastric emptying is overcome by most of these systems. However, there is still likely to be a considerable variability in the in vivo performance of the timed release systems by virtue of the variations in small intestinal transit time.

The pH of the GIT is acidic in the stomach and increases in the small and large intestine. This pH variation in different segments of GI has been exploited for colon-specific delivery. Coating the drug core with pH-sensitive polymers e.g. Eudragit® (methyacrylic acid-methylmethacrylate copolymers) has been successfully used for colon drug delivery in Asacol®, Salofalc®. These polymers are

insoluble in acidic media, but dissolves at a pH of 6 or more, thereby providing protection to the drug core in the stomach and to some extent in the SI releasing the drug in the colon. However, the pH of GIT is subject to both inter and intra individual variations, depending upon the diet, disease, age, sex and the fed/fasted state[19,20]. But due to the simplicity of the formulation of this device many marketed preparations utilize this approach. On prolonged use, these polymers may accumulate in the body so the use of biodegradable polymers is essential.

Osmotic systems independent of gastric residence time and metabolism by bacterial flora have also been developed for colon delivery of drugs. These systems are essentially timed release systems. OROS-CT systems developed by consist of a single or 5–6 units. These enteric coated push–pull units contain an osmotic push compartment and a drug compartment, both surrounded by a semipermeable membrane with an orifice[21]. As the unit enters the SI, the enteric coating dissolves and the osmotic push compartment containing an osmopolymer and an osmotic agent swells. Swelling of the osmotic push compartment forces the drug gel out of the orifice. These systems can be programmed to delay the drug release for varying durations .

Another strategy relies on the strong peristaltic waves in the colon that lead to a temporarily increased luminal pressure (pressure-controlled drug delivery). Pressure-sensitive drug formulations release the drug as soon as a certain pressure limit is exceeded. The pressure and the destructive force induced by peristaltic waves is certainly high in the distal part of the large intestine[22]. However, little is known about the reproducibility of this pressure and the duration of this high-pressure phase[23].

Biodegradable polymers have been used (a) as a linkage to form prodrugs with the drug moiety, (b) as a coating material to coat the drug core or (c) as an embedding media to embed the drug moiety in their matrices or hydrogels. Examples of such systems include azo polymers which are film forming and are used to coat the drug core. A synthetic polymer used to coat the drug capsule of insulin and vasopressin is a copolymer of styrene and hydroxyethyl methacrylate, cross-linked with 4-4′-divinylazobenzene and *N*,*N*′-bis (β-styrene sulphonyl)– 4,4′-diaminoazobenzene[24,25]. The azoreductase present in the colon degrades the coating and then releases the drug from the capsule. The use of such synthetic polymers, requires a more detailed toxicological studies.

The ability of natural polymers i.e. the polysaccharides, from algal origin (e.g. alginates), plant origin (e.g. pectin, guar gum) microbial origin (e.g. dextran, xanthan gum) and animal origin (chitosan, chondroitin) to act as substrates for the bacterial inhabitants of the colon together with their properties, such as swelling, film forming and their biocompatability, biodegradability invites their use as colon-carriers.

2. POLYSACCHARIDES

Carbohydrates are polyhdroxy carbonyl compounds which are also called ‘hydrates of carbon’. They consist of simple sugars having the empirical formula $C_nH_{2n}O_n$, where *n* isindicating that carbon atoms are in some way combined with water. In contemporary medicine and chemistry carbohydrate-based drug development has emerged as a highly promising and exciting area as a result there has been a significant increase in the number of reviews that address the general field of carbohydrate medicinal chemistry. This indicates the increasing importance of these new developments in carbohydrate-drug design, with a major focus on novel synthetic pathways and application in colon specific delivery.[26, 27]

Carbohydrate polymers of monosaccharide are found in abundance and are inexpensive thus attracting a lot of attention for targeting drugs to the colon. The use of natural polymers for colon-targeted delivery is based on the fact that anaerobic bacteria in the colon are able to recognize the various substrates and degrade them with the enzymes. The natural polymers has also attracted lot of attention because of their unique quality as they are stable in the gastric environment of the upper GIT and thus preferred for colon-targeted delivery. Carbohydrate polymers have created a great attention to pharmaceutical industries and researches for developing drug delivery system/technology to colonic region. Since they are easily available, cost effective and can be modified to more advanced form. The single carbohydrate is not as much effective as their modified form/combination with other polymer. Carbohydrate polymers like guar gum, pectin, chitosan, etc. can be cross-linked with suitable cross-linking agent. The cross-linked polymers control the release of drug in a desirable manner. Problem encountered with the use of polysaccharides is their high water solubility. An ideal approach is to modify the solubility while still retaining their biodegradability.

2.1 Chitosan

Chitosan is a high molecular weight, polycationic polysaccharide derived from naturally occuring chitin by alkaline deacetylation[28]. Chemically, it is a poly (*N*-glucosamine). Chitosan has favourable biological properties such as nontoxicity, biocompatability and biodegradability[29]. Chitosan however is soluble in dilute acid and precipitates at a pH above 7. Because of the solubility of chitosan at low pH ranges, its successful use in colon-specific delivery requires an enteric layer over the chitosan which would protect it against the acidity of the stomach. As the

formulation reaches the intestine, the pH increases and the enteric layer dissolves releasing the chitosan coated core. These cores are acted upon by microflora of the colon, degrading the chitosan and releasing the drug.

Chitosan capsules enteric coated with a layer of hydroxypropylmethylcellulose (HPMC) phthalate have been evaluated for colon delivery of drugs[30]. In vitro studies showed that the capsules loaded with a soluble dye, 5-(6)-carboxyfluorescein (CF) showed little release in simulated gastric juice for 2 h (transit time in stomach) and in artificial intestinal juice (next 4 h), but the presence of rat cecal contents (33%) in the dissolution fluid increased the release rate of the drug, CF from the capsules from 20 to 100% in the next 4 h. This suggests that the flora present in the rat cecal content may have produced enzymes for the degradation of chitosan or alternatively, the bacterial fermentation in the cecal contents may have decreased the pH of its contents and chitosan may have easily dissolved under acidic condition. In vivo studies carried out in wistar rats using insulin as the drug, showed improvement in absorption of insulin using these capsules. Also, the effect of absorption enhancers in increasing the absorption of insulin from these capsules was assessed.

Results were compared with the oral administration of insulin in a solution form and in a gelatin capsule form. The hypoglycemic effect and the plasma levels of insulin was assessed from time to time. The latter two formulations showed no hypoglycemic effect or peak insulin concentration. However, sharp peaks of plasma insulin concentrations and marked hypoglycemic effect was seen 6–12 h after the administration of oral chitosan capsules loaded with insulin and sodium glycocholate. Hypoglycaemic effect started 6 h after administration of capsule, thus showing that the absorption started in the large intestine. It was also seen that insulin absorption in the large intestine increased in the presence of absorption enhancers like sodium glycocholate. Comparing the effect of various absorption enhancers and protease inhibitors on the absorption of insulin showed that maximum increase in the absorption was caused by sodium glycocholate followed by aprotinin, soyabean trypsin inhibitor (STI), sodium oleate, bacitracin and *n*-dodecyl-β-d-maltopyranoside. Similarly, acceleration in the healing effect of R68070 (a new thromboxane synthase inhibitor) on 2,4,6-trinitrobenzene sulfonic acid-induced ulcerative colitis in rat using chitosan capsule as a carrier was compared to a carboxymethylcellulose (CMC) suspension of R68070[31]. The colitis in male wistar rats was not affected much with the administration of lower doses of both the above formulations. However, when the doses were increased a marked reduction in colitis was seen with R68070 when given in chitosan capsules rather than in CMC suspension leading to the same conclusion that these capsules have a good potential for being used as carriers in colon-specific drug delivery systems. Suzuki et al[32]prepared hard capsules of chitosan with enteric coating for the drug delivery specifically to the colon. The results shown by these capsules were also promising. Chitosan microspheres of sodium diclofenac were prepared by spray drying technique[33]. These microspheres were enteric coated with Eudragit L-100 or Eudragit S-100. Eudragit coating gave a pH-dependent release profile and the change in molecular weight of chitosan or use of different salt like chitosan glutamate could control the release rate of sodium diclofenac from the core. No release was seen in acidic pH for 3 h, but at higher pH Eudragit dissolved and swelling of chitosan started leading to continuous drug diffusion which completed in the next 4 h. Infra red studies revealed an ionic interaction between the amine group of chitosan with the carboxyl group of Eudragit, which provided another release controlling mechanism. Semisynthetic derivatives of chitosan i.e. chitosan succinate and chitosan phthallate were prepared by reacting chitosan seperately with succinic and phthalic anhydrides. Sodium diclofenac was dispersed in their matrices. In vitro studies showed that these matrices resisted dissolution under acidic conditions and showed improved dissolution under basic conditions, suggesting their suitability for colon-specific drug delivery systems. However, in vivo studies would be required to establish the suitability of these derivatives for colon-specific drug delivery.

The number fraction (%) of GlcNAc residues in the polymer chain is designated by the degree of acetylation that significantly influences the chitosan physico-chemical properties such as solubility, reactivity, biodegradability and cell response. Most of them are acid soluble, water-soluble derivatives (e.g. carboxylated derivative) can also be obtained. Chitosan is produced commercially by deacetylation of chitinin. It is hydrophilic, cationic and crystalline polymer that demonstrates film forming ability and gelation characteristics[34]. The ability of chitosan to prolong residence time in the gastrointestinal tract through mucoadhesion, and its ability to enhance absorption by increasing permeability have all been major factors contributing to its widespread evaluation as a component of oral dosage forms. Despite the outstanding scientific progress being made in the application of chitosan in drug delivery systems, no chitosan-based drug delivery systems have been launched to the market yet.

Wang et al.[35] prepared polyelectrolyte complex (PEC) of sodium cellulose sulfate (NaCS) and chitosan. FTIR data indicated that the NH_3^+ of the chitosan had reacted with the SO_4^- of the NaCS. NaCS–chitosan complex released about 80% of the drug in the SCF during 4 h. The study suggested that the NaCS–chitosan complex showed excellent behavior for colon specificity. The system could be a potential material for a colon-specific drug delivery system .Smoum et al[36]prepared chitosan–pentaglycine–

phenylboronic acid conjugate for colonic delivery of calcitonin. Three types of conjugates were prepared. In the first, 4-formylphenylboronic acid was directly linked to chitosan. The other two conjugates employed glycylglycine and pentaglycine spacers. Enzyme-inhibition assays toward trypsin and elastase, in the presence of the enzyme chitosanase, demonstrated a strong inhibitory effect for the chitosan–pentaglycine–phenylboronic acid conjugates, while no inhibitory effect was detected without chitosanase. The chitosan–pentaglycine–phenylboronic acid conjugate with the highest degree of substitution of 4-formylphenylboronic acid was able to decrease the salmon calcitonin degradation rate by trypsin. It is concluded that chitosan–pentaglycine–phenylboronic acid conjugates are a potential multifunctional, colon-specific vehicle for orally administered salmon calcitonin.

2.2 Pectins

Pectins are non-starch, linear polysaccharides extracted from the plant cell walls. They are predominantly linear polymers of mainly α-(1-4)-linked d-galacturonic acid residues interrupted by 1, 2- linked l-rhamnose residues. Pectin has a few hundred to about one thousand building blocks per molecule, corresponding to an average molecular weight of about 50 000 to about 180 000 .

These polysaccharides remain intact in the physiological environment of the stomach and the small intestine, but are degraded by the bacterial inhabitants of the human colon.[37]Being soluble in water; pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine. It was found that a coat of a considerable thickness was required to protect the drug core in simulated in vivo conditions[38]. So, the focus shifted to the development of such derivatives of pectin which were less water soluble but were degradable by the colonic microflora[39]. Calcium salts of pectin reduced their solubility by forming an 'egg-box' configuration .Amount of calcium in the formulation should be carefully controlled to ensure optimum drug delivery[40]. Matrix tablets of indomethacin were prepared with calcium pectinate and also with pectin. Releases of indomethacin from these matrices were studied in presence of (a) pectinolytic enzymes, (b) *Bacteroides ovatus* and (c) rat cecal contents[39]. The release of indomethacin from pectin-indomethacin tablets in 6 h was 0% in the absence of enzymes. The increase in percentage of pectinolytic enzymes in the dissolution medium increased the release of indomethacin. At 120 FDU/ml enzyme concentration, drug release was 100% within 2 h. When these tablets were studied in the presence of *B. ovatus* drug release was 12–21%. For calcium pectinate-indomethacin tablets release was only 16.2±2.2% after 72 h in absence of enzymes. In presence of pectinolytic enzymes the total amount of drug was released within 6 h. In the presence of rat cecal contents, release was about 60.8±15.7% as compared to 4.9±1.1% of the control medium without cecal contents. This study showed calcium salt of pectin as a promising colon drug targeting matrix since significant difference between indomethacin levels were observed in the presence of rat cecal content and control, at each time.

Further studies comparing the utility of compression coating technique to deliver two different types of drugs to the colon, firstly, a water insoluble drug, indomethacin and secondly, a water soluble drug, insulin as compared to plain matrix tablet technique[41].Calcium pectinate-indomethacin tablets of both types, i.e. compression coated and matrix tablets showed no release of indomethacin at a pH of 1.5 for 2 h. When these tablets were shaken at a pH of 7.4 a drug leak was seen in plain matrix tablets but not in compression coated tablets. In the presence of pectinolytic enzymes, a sudden release of indomethacin was seen in both, the types of tablets but the rate and the percentage of release were lower (only 57.6±2.5%) in compression coated tablets as compared to plain matrix tablets (74.2±4%) after 12 h. Two types of enteric coated calcium pectinate matrix tablets were formulated by Adkin et al.[42] one using pectin as a binder (CaP/P) and the other using guar gum as a binder (CaP/GG). Scintigraphic evaluation of gastrointestinal transit and disintegration in ten healthy human volunteers showed that the intact tablets arrived in the colon where the complete disintegration occurred for both the formulations. However, CaP/GG tablet showed slower disintegration as compared to CaP/P tablet. The time and location of complete tablet disintegration was more reproducible with CaP/P tablet as compared to CaP/GG tablet. Methoxylated pectin system where the acid group had been 70% methoxylated, applied as a compression coat was found to be protective to the core tablet during conditions mimicking mouth to colon transit[38]. Another derivative of pectin, amidated pectin was considered for colonic delivery because of its biodegradability, higher tolerance to pH variations and fluctuations in calcium levels. They were susceptible to enzymatic breakdown .Pectin 920 and 4200 having different degrees of substitution were evaluated[43]. However, the release of paracetamol from both these substituted polysaccharides in vitro was quite high and it was found that they were not able to resist the drug release before arrival into the colon. Inclusion of calcium as cross-linking agent increased the viscosity of amidated pectin gels to a maximum. Pectin 920 formed strong gels, which showed drug release retarding properties. This pectin might be of value in colonic delivery either alone or in combination, possibly in the form of a coating.

Many of the colon-specific drug delivery systems developed are single unit systems. A drawback of single unit system is that

they may exhibit a delay at the ileocecal junction, leading to drug loss prior to entry into the colon. This can be avoided with the use of multiparticulate systems. Multiple unit systems like pellets have been shown to spread out on their entry to the colon[44]. This increases the surface area causing rapid bacterial breakdown and is followed by a rapid drug release and thereby improved absorption. So, multiparticulate system consisting of hydrogel beads based on amidated pectin as matrices were developed.In another attempt to overcome the drawback of high solubility of pectin, mixed films of pectin with ethyl cellulose were investigated as a coating material for colon-specific drug delivery. Working on similar grounds, the leaching of pectin from the mixed films prepared with Aquacoat® ECD 30, Surelease clear®, Eudragit® RS30D or Eudragit® NE 30Dwas studied in presence and absence of pectinolytic enzymes[44]. Pectin was quickly released from all the films except the mixed films of pectin – Eudragit® RS. However, these films showed significantly increased leaching of pectin in the presence of pectinolytic enzymes. Further studies using aqueous dispersions of Eudragit® RL, NE, RS, Aquacoat and surelease mixed with pectin/calcium pectinate and showed the decrease in release rate of theophylline in presence of pectinolytic enzymes as compared to release rate in absence of enzymes[44,45]. This was explained on the basis that hydrophilic polymers like pectin and calcium pectinate incorporated in water soluble coating, hydrated, forming pectin channels through which the hydrophilic drug could diffuse. Enzymatic degradation of pectin from these films suppressed the hydration of these films, which then restructure. Restructuring plugs the possible pores and slows down the drug release. This showed that such systems were unsuccessful for targeting drugs to the colon[46].An inter-polymer complex of pectin with chitosan was prepared by Meshali et al[47]. In vitro studies of the potential of this mixture for colon-specific delivery was further investigated by Fernandez-Hervas and Fell (1998)[48]. They used indomethacin and paracetamol as model drugs to represent poorly soluble and soluble drugs. In vitro studies using pectin and chitosan as a compression coat showed that this coat could offer greater protection at a lower coat weight in the upper GIT than pectin alone (in which a substantially thick coat is required for protection). Results were better with water insoluble drug as compared to water soluble drug.Combination of pectin, chitosan and HPMC films have also been studied for their potential as colon-specific drug delivery systems[49,50]. These films having pectin, chitosan and HPMC in the ratio 3:1:1 were found to be insoluble and showed different degrees of swellings on varying the concentration of pectin and chitosan. These films were degradable by the pectinolytic enzymes. Core tablets were coated with these mixed films and a radioactive marker technetium-99 was introduced into the tablet by drilling a hole in the centre. This hole was sealed with pectin and chitosan followed by enteric coating of the prepared tablet. In vitro studies of these tablets carried out in 0.1 M HCl (for 2 h) and in Sorensen's buffer (for 3 h) showed minimal release of the radioactive marker. However, in the presence of pectinolytic enzymes the release was almost 100% in the next 2.5 h. In vivo studies were done in human volunteers. The most desirable property of pectin is that it is gastric resistance and degradable by colonic bacteria. Pectin contains varying degrees of methyl ester substituents, which depend upon the plant source and preparation. The solubility and gelation of pectin are also affected by the methyl groups. Higher methoxy pectins require a less soluble solids and a pH about 3 to form gels. While the low methoxy pectins require the presence of a controlled amount of calcium ions for gelation and need neither sugar nor acid. When the degree of esterification is less than 50%, pectins form rigid gels by reacting with calcium salts or multivalent cations, which crosslink the galacturonic acids of the main polymer chains. The methoxy content of pectin affect the drug release from polymer matrix. Muhidinov et al.[51] prepared new microcapsular system delivery from pectins obtained from various sources, with different molecular weight and the degree of esterification for colon delivery. Predsinolone was studied as model drug. They investigated the release kinetics of poor water soluble drug from the pectin microcapsules. The highest value of drug dissolution/diffusion number was obtained for microcapsule from high methoxylated apple pectin in the presence of anionic surfactants and calcium ions, rather than for the systems of highly charged citrus pectin. Capsules prepared by the use of ethyl acetate also showed retarded drug release, however the amount of drug encapsulated was much less than those from other emulsion systems. The study suggested the application of biodegradable pectin polysaccharides in the production of vastly diverse drug carrier systems for colon-specific drug delivery.

2.3 Guar Gum

Guar gum derived from the seeds of *Cyamopsis tetragonolobus* is a naturally occuring galactomannan polysaccharide. It is made up of a linear chain of β-d-mannopyranose joined by β-(1-4) linkage with α-d-galactopyranosyl units attached by 1,6-links in the ratio of 1:2.

Guar gum contains about 80% galactomannan, 12% water, 5% protein, 2% acid insoluble ash, 0.7% ash and 0.7% fat. Guar gum hydrates and swells in cold water forming viscous colloidal dispersions or sols[52]. This gelling retards the drug release from the tablets[53]. Guar gum is being used to deliver drug to the colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine[54-56].Guar gum based matrix tablets of dexamethasone and other antinflammatory agents have shown very encouraging results as colon-carriers. Matrix tablets of dexamethasone and budesonide were prepared using 60.5% w/w of guar gum in the tablet[57]. The study showed negligible drug release in simulated gastric and intestinal fluid whereas in simulated

colonic fluid significant increase in drug release was observed. The study demonstrated that the galactomannanase (>0.1%) accelerated dissolution of dexamethasone and budesonide from guar gum matrix tablet. The extent of drug dissolution depended on concentration of galactomannanase. The high molecular weight of guar gum provides a highly viscous solution in cold water. Guar gum is soluble in cold water, hydrate rapidly to produce viscous pseudoplastic solutions that although shear-thinning generally have greater low-shear viscosity than other hydrocolloids. The gelling of guar gum retards release of the drug and it is susceptible to degradation in the colonic region. The modified products are having better swelling and enzymatic degradation properties. Guar gum was reacted with glutaraldehyde under acidic conditions to obtain different products with increasing crosslinking densities. To prevent premature drug release, guar gum with low swelling index was manufactured by crosslinking it with trisodium trimetaphosphate, glutaraldehyde, which was capable of delivering drug to the colon.

Further investigations were also conducted to evaluate the suitability of guar gum as a carrier in colonic drug delivery. In one study, matrix tablet of indomethacin with guar gum were prepared[58]. These tablets were found to retain their integrity in 0.1 M HCl for 2 h and in Sorensen's phosphate buffer (pH 7.4) for 3 h. releasing only 21% of the drug in these 5 h. However, in presence of 2% rat cecal contents the drug release increased and further increased with 4% concentration of cecal contents. The drug release improved to about 91% in 4% cecal content medium after enzyme induction of rats. This study suggests the specificity of these matrices for enzyme trigger in the colon to release the drug. In the absence of enzyme system the guar gum swells to form a viscous layer that slows down the seeping of the dissolution fluid into the core. The initial 21% release can be attributed to the dissolution of indomethacin present on the surface of the tablet.In another in vivo study, matrix tablets containing around 77% guar gum were loaded with technetium-99m-DTPA as tracer and scintigraphs were taken at regular intervals in six healthy human male volunteers[59]. These tablets were found to remain intact releasing only small amount of tracer in the stomach and the small intestine. However, bulk of the tracer was released in the ascending colon thereby suggesting the enzyme trigger degradation by colonic bacteria. Guar gum has also been evaluated as a compression coating to protect the drug core of 5-ASA in upper GIT[60]. The tablets coated with 300, 200 and 150 mg of guar gum showed cumulative mean drug release percentages of 5.98±0.70, 8.67±0.35 and 12.09±0.29 respectively after 26 h while tablets coated with 125 mg guar gum disintegrated within 5 min in simulated gastric fluid. Cores with guar gum coat as high as 300 and 200 mg could not successfully release the drug in presence of rat cecal contents even in 26 h as drug release was 23.85±3.13 and 63.43±6.30%, respectively. However, the formulation with 150 mg of guar gum as a coating showed 95.51±1.50% of 5-ASA release in presence of rat cecal contents after 26 h. Percent drug release from tablet increased considerably from 11th hour and the tablets were completely disintegrated in 26 h. The results of drug release studies on compression coated tablets suggested that the thickness of guar gum coating in the range of 0.61–0.91 mm was sufficient to deliver the drugs selectively to the colon.

Pinhassi et al [61]investigated that tethering of both FA and methotrexate (MTX) to arabinogalactan (AG), a highly branched natural polysaccharide resulted in a targeted biomacromolecular nanovehicle with unusual water solubility. This nanovehicle can differentially deliver a cytotoxic cargo into FR-overexpressing cells. They demonstrated a target-activated release mechanism. The C5-FRR cells were incubated in the presence of excess free FA (50 μM). In this case, a 6.3-fold higher cytotoxicity was also obtained for C5-FRR cells incubated in FA-free medium relative to cells incubated with excess FA. The average IC50 values obtained for C5-FRR cells in FA-lacking medium and in medium supplemented with 50 μM FA were 400 ± 13 and 2500 ± 65, respectively. The FA-AG-GFLG-MTX drug conjugate showed 6.3-fold increased cytotoxic activity to FR-overexpressing cells compared to their FR-lacking counterparts.

2.4 Dextrans

Dextrans are a class of polysaccharides with a linear polymer backbone with mainly 1,6-α-d-glucopyranosidic linkages. They are obtained from bacterial cultures of *Leuconostoc mesenteroides* NRRL B-512. These glycosidic linkages are hydrolysed by moulds, bacteria and also by the mammalian cells[62-64]. Dextranases are the enzymes which hydrolyse these glycosidic linkages. Dextranases activity of the colon is shown by anaerobic gram-negative intestinal bacteria especially the bacteroides[65]. Dextran has also been found to be degraded in human feaces due to bacterial action[66].

Dextran is water soluble and easily functionalized through its reactive hydroxyl chemistries. Biodegradation occurs through natural enzymatic splitting of polysaccharides bonds by dextran–1, 6-glucosidase found in liver, spleen, lungs, brain and by dextranases expressed by bacteria in the colon. The dextran resists protein and lacks nonspecific cell binding, which has increased its use as a biomaterial. The hydroxyl functionality for chemical modification of dextran and relatively low cost, availability has increased the utilization of dextran in the field of polysaccharide polymer conjugates for biomaterials.Various drug-dextran prodrugs in which the drug molecule is linked to the polar dextran

macromolecule remain intact and unabsorbed from the stomach and the small intestine but when the prodrug enters into the colonic microflora containing as much as 10^{11}*Bacteroides* per gram[67]. It is acted upon by dextranases which cleave the dextran chain randomly and at the terminal linkages releasing the drug, free into the colon. Increasing interest is being focused on dextran prodrugs. First attempt was carried out by who conjugated naproxen to dextran by ester linkage[16]. Dextran ester prodrugs of ketoprofen and naproxen using dextran with molecular weight (M.W.) 10 000–500 000 were shown to release the drug specifically in the colon region of pig[68, 69]. The release of naproxen was upto 17 times higher in the cecum and colon homogenates of pig than in control medium or homogenates of SI. A series of prodrugs, naproxen-dextran, ketoprofen-dextran and ibuprofen-dextran have been tested in vitro and in vivo in pigs. They postulated this prodrug system as a potential system for site specific delivery showing high bioavailability of the drug but still no absorption of the prodrug into the circulation. Also, this system could provide protection to the drug in the upper GIT and selective regeneration in the cecum/colon. This approach delivers drug specifically to the colon and can be used for colon targeting.

In another study, dextran with molecular weight 72 600 were used to deliver to the colon[70,71].Methyl prednisolone and dexamethasone prodrugs with dextrans were prepared. Since the glucocorticoids did not have a functional group for attachment of dextrans, these were attached to dextrans using a spacer molecule[71, 72]. It was found that dextran conjugates showed little hydrolysis in upper GIT contents, but were degraded rapidly in the cecal and colonic contents. These polymeric prodrugs were much more effective than the parent drug itself when tested in colitis-induced rats[73]. Jung et al.[74] prepared dextran prodrugs of 5-ASA. This prodrug was stable and no 5-ASA was released in contents of SI of rats. Even the presence of diluted cecal contents in the dissolution medium increased the release of 5-ASA from the prodrug. Apart from prodrug approach biodegradable dextran hydrogels using diisocyanate as a cross-linking agent) have been found to be fully degradable by dextranases in vitro and in vivo in the rat cecum[75, 76]. These have also been found to be completely degradable in the human colonic fermentation model though, the fermentation of the gels started only after around 24 h. This would however, cause a delay in drug release which would take place in the distal colon where conditions for absorption are very reduced.

pH-sensitive dextran hydrogels were prepared by activation of dextran with 4-nitrophenyl chloroformate followed by conjugation of activated dextran with 4-aminobutyric acid and cross-linking with 1,10-diaminodecane[77]. Bovine serum albumin (BSA) was loaded in these dextran hydrogel discs by immersing the discs in BSA solutions. After equilibrating in the BSA solution for 5 days, the discs were washed and dried. The release rate of bovine serum albumin from these hydrogels discs was primarily determined by the swelling extent which in turn depended upon the content of carboxylic acid and the degree of cross-linking. The release rate was enhanced by the addition of dextranase in the dissolution media.A novel pH-sensitive and biodegradable composite hydrogel, based on a methacrylated and succinic derivative of dextran, named Dex-MA-SA, and a methacrylated and succinic derivative of α,β-poly(N-2-hydroxyethyl)-dl-aspartamide (PHEA), named PHM-SA was prepared. The goal was to obtain a colon-specific drug delivery system, exploiting both the pH-sensitive behavior and the colon-specific degradability. The hydrogel was prepared with a suitable ratio between the polysaccharide and the polyaminoacid. It was characterized regarding its swelling behavior in gastrointestinal simulated conditions, chemical and enzymatic degradability, interaction with mucin, and cell compatibility on cells. In vitro drug release studies, performed using 2-methoxyestradiol as a model drug, showed that Dex-MA-SA/PHM-SA based hydrogel was able to release the drug in simulated intestinal fluid, especially in the presence of dextranase and esterase. Furthermore, the potential mucoadhesive behavior of the hydrogel promoted drug release in the site of action for a prolonged time. The obtained results show that this polysaccharide/polyaminoacid hydrogel is potentially useful for the oral treatment of colonic cancer.

In another approach, Glutaraldehyde cross-linked dextran capsules were used for colon-specific drug delivery. These capsules were loaded with hydrocortisone and drug release studies were conducted in vitro. Ten percent of drug was released in initial 3 h and only about 35% in 24 h at pH 5.4. Addition of dextranase enzyme after 24 h resulted in a rapid degradation of the capsule leading to fast and complete release of hydrocortisone. However, these results reflect only an experimental condition and not the in vivo situation.

Larrosa et al[78] explored the efficacy of different resveratrol prodrugs and pro-prodrugs to ameliorate colon inflammation in the murine dextran sulfate sodium (DSS) model. Mice fed with a very low dose (equivalent to 10 mg for a 70 kg-person) of either resveratrol-3-O-(6′-O-butanoyl)-β-d-glucopyranoside or resveratrol-3-O-(6′-O-octanoyl)-β-d-glucopyranoside did not develop colitis symptoms and improved 6-fold the disease activity index (DAI) compared to resveratrol. The study indicated that these pro-prodrugs exerted a dual effect: (i) prevention of rapid metabolism of resveratrol and delivered higher quantities of resveratrol to the colon and (ii) reduction in mucosal barrier imbalance and prevented diarrhea, which consequently facilitated the action of the delivered resveratrol in the colon mucosa.

Colon degradable dextran fatty acid esters which were film forming and insoluble in gastric and small intestinal fluids were synthesized for colon drug delivery. Out of these esters lauroyl dextran esters with molecular weight of approximately 250 000 and degree of substitution ranging from 0.11 to 0.3 were found to be suitable for colon drug delivery as film coatings. Initial studies carried out in vitro with lauroyl dextrans esters having degree of substitution between 0.12 and 0.40 and using theophylline as the drug showed that release rate was inversely proportional to the amount of ester applied on the coating[79]. Addition of dextranase degraded the coating releasing the drug. However, further studies showed that dispersion of lauroyldextrans were not suitable as degradable coating material, as they did not display ideal zero order dissolution before and quick disintegration after enzyme addition[80].

2.5 Inulin

Inulin is a naturally occurring polysaccharide found in many plants, such as onion, garlic, chicory, artichoke[81]. Chemically, it consists of β 2-1 linked d-fructose molecules, having a glucosyl unit at the reducing end[82].Inulin is not hydrolysed by the secretions of the human digestive tract. Bacteria present in the colon especially *bifidobacteria*, which constitute up to 25% of the normal gut flora in man are known to ferment inulin[83, 84].

To overcome the poor film forming property and to control the swelling of inulins, they have been evaluated for colon-targeting in combination with synthetic film forming polymers. The mixed films thus prepared resisted degradation in the upper GIT and fermented in the colon by *Bifidobacteria* and *Bacteroides*. Vervoort and Kinget[85]incorporated highly polymerised inulin in Eudragit RS films which were degraded in human fecal medium. Also, the permeability of these membranes increased significantly after incubation in the fecal medium. A series of studies were carried out on chicory inulin[86]. Methacrylated inulin was synthesised and aqueous solutions of Methacrylated inulin upon free radical polymerisation, were converted to cross-linked hydrogels. Rheological studies and characterization of there hydrogels showed that higher substituted inulins had better network and higher mechanical strength[87]. These hydrogels were then studied for their swelling properties and degradation in vitro[88, 89]. Degradation studies carried out in the presence of inulinase showed that increasing enzyme concentration and incubation time degraded inulin faster. However, increasing the substitution on inulin molecules resulted in stronger hydrogels with less enzyme diffusion thereby less degradation.

2.6 Chondroitin Sulphate

Chondroitin sulphate is a mucopolysaccharide found in animal connective tissues especially in cartilage. Chemically, it consists of d-glucuronic acid linked to *N*-acetyl-d-galactosamide which is sulphated at C-6[90].

Chondroitin sulphate is degraded by the anaerobic bacteria of the large intestine mainly by Bacteroides thetaiotaomicron and B. Ovatus[91]. Such a degradation profile suggests the use of chondroitin sulphate as a drug carrier to deliver drugs especially to the large intestine where *bacteroides* are found in abundance. However, the high water solubility of chondroitin sulphate is disadvantageous. There was 100% release of indomethacin within 1 h of dissolution test using chondroitin sulphate alone as a carrier. To overcome this difficulty, cross-linked chondroitin was developed as a drug carrier for colon-specific delivery[92]. Chondroitin sulphate was cross-linked with 1, 12-diaminododecane via dicyclohexylcarbodiimide activation. Cross-linked chondroitin sulphate was used to form a matrix tablet with indomethacin. Release of indomethacin from this tablet was studied in the presence of rat cecal contents as compared to release in phosphate buffer saline. A significant difference in drug release was observed after 14 h in the two dissolution media. Also, different degrees of cross-linked chondroitin sulphate were used to study their effect on drug release from the matrices. The cumulative percent release of indomethacin from cross-linked chondroitin matrix tablet showed that release was increased in the presence of rat cecal contents. Studies on rat cecal contents with various cross-linked chondroitin sulphate showed greater cumulative drug release when cross-linking was less and as cross-linking increased the cumulative release decreased i.e. a linear relationship was found between the degree of cross-linking of polymer and the amount of drug released in rat cecal content. This suggests that the drug release in the colon can be controlled by adjusting the relative amount of different cross-linked chondroitin sulphate in the matrices.

2.7 Amylose

Amylose is a polysaccharide from plant extracts and a component of starch. It consists of d-glucopyranose residues linked by α-(1→4) bonds. It is a poly (1, 4′-α-d–glucopyranose). This naturally occurring polysaccharides possesses the ability to form films. These films are water swellable and are potentially resistant to pancreatice α-amylase[93] but are degraded by colonic bacterial enzymes [94, 95].

Amylose-Ethocel coating system resistant to gastric acid and small intestinal enzymes, but degradable by colonic bacteria were prepared and evaluated in vitro for their potential as colon drug carrier. Varying concentrations of Amylose and Ethocel in the

form of aqueous dispersions were used to coat 5-ASA pellets. A coating formulation comprising Amylose and Ethocel in the ratio of 1:4 w/w showed optimum drug release retarding properties in gastric and intestinal fluids[96, 97]. This coating with amylose: ethycellulose in ratio of 1:4 could suppress the in vitro release of 5-ASA from coated pellets in simulated gastric and small intestinal media over a period of 12 h and was fermented in simulated colonic environment (containing mixed fecal bacteria of human origin) releasing the drug in 4 h.Further, the same group found that this amylose–ethycellulolse coating (1:4) was also suitable to deliver a highly water soluble drug glucose to the colon[98, 99]. Cores were made using glucose and Avicel® PH 101 (microcrystalline cellulose). To retard glucose solubilization, additional binders were added to the cores and secondly, amylose fraction in coating mixture was increased. As the thickness of the coat was increased, the glucose release decreased. Pellets core containing 20% glycerol monostearate, 50% glucose and 30% Avicel PH 101 coated with the Amylose–Ethocel® mixture (1:4) with 6.8% amylose concentration could resist glucose release in upper GIT conditions. In vitro fermentation studies in 5% mixed human fecal slurry showed an early release of glucose from these coated pellets. In vivo studies carried out in healthy human volunteers using gamma scintigraphy and breath samples for CO_2 showed that a delay of 2.7 h occurred between arrivals of pellets to the cecum and their significant breakdown. The release of glucose occurred over a period of time indicating that the site specific delivery in addition had a sustained release profile[99].Organic solvent based amylose–ethylcellulose films have also been evaluated as potential coatings for colonic drug delivery. Varying the concentration of amylose and ethylcellulose in the films could vary the drug release rate from these films[100]. 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 for delivery of 5-ASA pellets to the colon by this group[101]. The rate of release was inversely proportional to the thickness of the coat and also influenced by the amount of amylose present in the film. It was found that a film coating containing amylose and ethylcellulose in a ratio of 1:1 and 15% total weight gain after coating, could resist release of 5-ASA in upper GIT and gave a rapid drug release in simulated colonic condition. These films are believed to offer promises in the processing of drug molecules that are thermolabile and/or sensitive to water for colonic delivery.

2.8 Cyclodextrins

Cyclodextrins are cyclic oligosaccharides. They consist of 6–8 glucose units linked through α-1, 4′-glucosidic bonds.

Cyclodextrins are neither hydrolysed nor absorbed from the stomach and small intestine. However, in the colon the vast microflora present breaks these into small saccharides and thus are absorbed in the large intestine[102, 103]. This property of being able to be degraded by colonic bacteria especially *Bacteroides* led to its use as a colon targeting carrier. Ester conjugates of biphenylyl acetic acid with β-cyclodextrin released the drug preferentially when incubated with rat's cecal contents and almost no release was observed on incubation with contents of stomach and small intestine[17]. Studies were carried out for the prodrugs of α, β and γ cyclodextrins with biphenylyl acetic acid (BPAA) for colon-specific delivery[104]. Both ester and amide type prodrugs were prepared and in vivo release studies conducted in rat. Studies on ester prodrug showed that a large portion of the prodrug was recovered intact from stomach and only negligible amount of free BPAA was present. In the cecum and colon BPAA was produced and absorbed into the blood, suggesting that ring-opening hydrolysis takes place in the cecum and colon and the resulting small saccharide ester conjugates are rapidly hydrolysed to BPAA. These results were in accordance with the in vitro studies. The amide prodrug, however, in the in vivo studies was observed to be intact in the entire GIT but was hydrolyzed to BPAA-maltose conjugate in the cecum or colon. After 6 h of dosing, whole of the prodrug moved to the colon and intact prodrug and maltose conjugate were present in colon. Neither the prodrugs, nor BPAA-maltose, nor BPAA were present in the blood nor urine suggesting that hydrophilic carbohydrate conjugates are less absorbed from rat GIT. These observations indicated that the cyclodextrins prodrug passes intact from the rat stomach and small intestine but are subjected to ring opening process in the cecum and colon.

2.9 Alginates

Alginates are a linear polymer which have 1-4′ linked-β-d-mannuronic acid and α-l-guluronic acid residues arranged as blocks of either type of unit or as a random distribution of each type Alginates do not gel since they have poly(l-gluronic acids) which are rigid, Ca^{++} ions induce gelation.

The gel formation of polysaccharides in the gastric medium retards drug the release from the core. The presence of calcium ions is necessary since alginates do not gel because they have rigid poly (l-guluronic acids), which gel in the presence of calcium ions. Alginate gelation takes place when divalent cations (usually calcium ions) interact ionically with blocks of guluronic acid residues, resulting in the formation of a three-dimensional network that is usually described by an 'egg-box' model. It is the ion exchange process between sodium and calcium ions that is supposed to be responsible for the swelling and subsequent degradation of sodium alginate in the colon. The swelling and

mechanical properties of alginate, produced by ionic crosslinking with cations, depend on ionic properties. For example, monovalent cations and magnesium ions do not induce gelation whereas barium ions produce stronger beads than calcium ions[105]. Calcium alginate beads were made as cores and 5-ASA was spray coated on them. These beads were coated with different percentages of enteric coating polymer and/or sustained release polymer[106]. Aquacoat is a pH independent polymer which is insoluble in both acidic and intestinal fluid and Eudragit L-30D dissolves above pH 5.6. A system was formulated by coating 5-ASA calcium alginate beads with aquacoat (4% w/w) followed by 6% w/w coat of Eudragit L-30D. The Eudragit layer protected the coat in acidic medium but dissolved in the basic media. There, the rate of drug release was controlled by aquacoat. When drug loaded calcium aliginate beads swell sufficiently (osmotic gradient) to exceed the strength of the outer sustained released coat, the film bursts to release the drug. Such a system delivers drug to the distal intestine with minimal initial leak and gives sustained release in the colon. Also alginate beads coated with dextran acetate were prepared. These beads showed minimal drug release in the absence of dextranase but significant drug release was seen in presence of dextranases in vitro[107].

2.10 Locust Bean Gum

Locust bean galactomannan were found to be soluble in water. Cross-linked galactomannan however led to water-insoluble film forming product showing degradation in colonic microflora. However, dissolution study performed on theophylline tablets coated with cross-linked galactomannan showed the mechanical instability of these coatings in the dissolution media thereby suggesting the nonsuitability of such films as colon carriers[80].

3. POLYSACCHARIDE MODIFICATION

The modification/substitution in carbohydrate polymers leads to enhancement in targeting efficiency of therapeutic drug candidates. Substitutions in general greatly influence the physicochemical behavior of polysaccharides[108]. The modification can be brought by grafting of some polymers (PVA grafted guar gum), chemical reaction, (amidation of pectin, acetylation of guar gum) or derivative formation (succinic acid derivative of dextran), etc[109-111].

The colon specific delivery systems based on a single polysaccharide do not efficiently permit targeted release. The pH and transit time can vary depending on the individual and the particular disease state. Drug release can be premature or even non-existent in these cases. The combination/chemically modified forms of polysaccharides eliminated the drawbacks associated with the use of single polysaccharide. The industrial researches are going on with the use of mixtures of polysaccharide and their structurally/chemically modified forms. In this review emphasis is given on the application and properties of combination/modified forms of carbohydrate polymers employed for colon specific delivery [116,117].

3.1 Carbohydrate Mixtures

3.1.1 Cellulose derivatives

Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most common organic compound on the earth[118]. About 33% of all plant matter is cellulose (the cellulose content of cotton is 90% and that of wood is 40–50%). Cellulose consists of a linear chain of several hundred to over ten thousand d-glucose units which in contrast to starch are β-1,4 linked. This β-1, 4 linkages make cellulose linear, highly crystalline and indigestible for humans[119-121]. Since cellulose is not absorbed systemically following oral administration, it has little toxic potential and is thus also a generally recognized as safe (GRAS) listed material. Cellulose is one of the most important pharmaceutical excipients and food additives. Cellulose is a frequently used as tablet excipients[122,123].Cellulose esters can be distinguished into two categories, non-enteric and enteric. Non-enteric esters, like cellulose acetate, cellulose acetate butyrate (CAB) and cellulose acetate propionate do not show pH-dependent solubility characteristics and (with no commercial exceptions) are insoluble in water. The non-enteric cellulose esters can be used to sustain drug release from oral delivery systems either by formation of a matrix or an insoluble but permeable film. Enteric esters are those, such as cellulose acetate phthalate (CAP) or hydroxypropylmethyl cellulose phthalate (HPMCP) which are insoluble in acidic solutions but soluble in mildly acidic to slightly alkaline solutions. The dissolution pH of these polymers depends upon the degree of esterification. The different HPMCP types dissolve within the pH range of 4.5–5.5 that is the pH values in the upper small intestine portion of GIT. While CAP dissolves at somewhat more neutral pH (pH >6) which indicate that drug release from CAP coated dosage forms occurs in the jejunum, the mid-part of the small intestine[124, 125].

3.1.2 Cellulose acetate phthalate

CAP is used as an enteric polymer and for oral controlled release formulations. It is a cellulose polymer where about half of hydroxyls groups are esterified with acetyls, a quarter are esterified

with one or two carboxyls of a phthalic acid, and the remainder is unchanged. CAP has pH dependent solubility and has been used for several decades as pharmaceutical excipients. CAP coated formulations are resistant to acidic pH (gastric fluids), but easily soluble in mildly alkaline medium (intestine). The pH dependent solubility of CAP is mainly determined by the degree of substitution, i.e. the average number of substituent groups bound to an anhydroglucose unit, as well as by the molar ratio (acetyl and phthaloyl groups). These two important structural properties CAP are dependent on the method employed for its synthesis[126].

3.1.3 HPMC

Hypromellose (INN), (HPMC) is a solid, and is a slightly off-white to beige powder in appearance and may be formed into granules. Hypromellose in an aqueous solution, unlike methylcellulose, it does not exhibit thermal gelation property. The methoxy content determines the inflexibility/viscosity of HPMC solution. The higher methoxy content, the more viscous or less flexible will be the solution[120].

3.1.4 HPMCP

HPMCP was introduced into the market in 1971 as an enteric coating polymer. Shin estu chemical company has made 3 enteric polymers available commercially. These are derived from hydroxyl propyl methyl cellulose N.F. by esterification of with phthalic anhydride and are marketed as HPMCP 50, 55 and HP-55-S HPMCP is the trade name for hydroxyl propyl methyl cellulose phthalate. These polymers dissolve at loser pH 5–5.5 than CAP or acrylic polymers[121].

3.1.5 Amylose–ethylcellulose

Amylose is degraded by colonic bacteria and is resistant to, among others things, pancreatic enzymes. At body temperature, the amorphous amylose is in the vitreous state and is insoluble in gastric and duodenal juices. The use of amylose as macromolecules to construct matrix systems or coatings led to swelling of the systems and, hence, to premature drug release[127]. Combination with ethylcellulose makes it possible to control this swelling and release of the drug[128]. Delayed-release dosage forms have been prepared using an active substance mixed with amorphous amylose, the mixture being coated with an inner coating of amorphous amylose and an outer coating of a film-forming cellulose or acrylic polymeric material or a mixed coating composed of amylose and a film-forming cellulose or acrylic polymeric material. Allwood et al.[129] also suggested applying the same concept using vitreous amylose as matrix and coating agents. A combination of a complex such as butan-1-ol amylose and an aqueous dispersion of ethylcellulose (Ethocel®) at a ratio of 1:4 (w/w) has been used to coat pellets containing salicylic acid and glucose with Eudragit RS and RL[97,98]. Some in vitro studies and others conducted in humans have demonstrated that glucose was released in the colon using this kind of coating[99]. Recently, Newton and Siew[130] developed a coating system made of amylose and a water-insoluble film-forming polymer (in the weight ratios 3:2 and 2:3) using an organic solvent, while Newton and Leong [131] patented a similar system, using a mixture of an amylose-alcohol complex and ethylcellulose, to coat tablets. The interesting in vitro results need to be confirmed *in vivo*.

3.1.6 Pectin–HPMC

Ugurlu et al prepared compression coated tablets of nisin using different combinations of pectin/HPMC. Pectin alone was insufficient to protect the nisin containing core tablets. At the end of the 6 h 40% degradation was observed for 100% pectin tablets. HPMC addition required to control the solubility of pectin, a 5% increase in HPMC ratio in pectin/HPMC mixture provided a 2 h lag time for nisin release. Eighty percent pectin–20% HPMC appeared to be an optimum combination for further evaluation. Tablets maintained their integrity during the 6-h dissolution test, approximating the colon arrival times. The polymer hydration affected the enzymatic degradation of pectin[132]. The pectin–HPMC compression coated tablet labelled with 4MBq (99m)Tc-DTPA was developed by Hodges et al[133]. Authors studied the in vivo behavior of tablet in six healthy male volunteers three in the ascending colon (AC) and three in the transverse colon (TC). Prolonged residence at the ileocaecal junction (ICJ) was assumed to have increased hydration of the hydrogel layer surrounding the core tablet. Inadequate prior hydration of the hydrogel layer preventing access of pectinolytic enzymes and reduced fluid availability in the TC may have retarded tablet disintegration and radiolabel diffusion[132].

3.1.7 Pectin-hydroxypropylmethylcellulose

Matrix tablets have been prepared by direct tableting from mixtures of high methyl ester pectins/hydroxypropylmethylcellulose and from mixtures of high methyl ester pectins/hydroxypropylmethylcellulose/gelatin[134-136]. Thick layers of coatings made of high methyl ester pectins by direct tableting have made it possible to considerably delay the release of tracers from the tablets in dissolution media.Studies on conventional coatings have been conducted using pectin/chitosan/hydroxypropylmethylcellulose mixtures. Used as tablet coatings, these are also able to delay drug release before it is completely degraded in the colon. However, it has been

demonstrated that the release of the drugs from these formulations begins in the small intestine in humans[137,138].

3.1.8 HPMC–NaCMC

Chaudhry et al. prepared microporous bilayer osmotic tablet of dicyclomine hydrochloride and diclofenac potassium for colon specific delivery. The HPMC and Na-CMC were used as osmogen. The number of pores was dependent on the amount of the pore former in the semipermeable membrane. In vitro dissolution results indicated that system showed acid-resistant, timed release and was able to deliver drug at an approximate zero order up to 24 h[139].

3.1.9 Chitosan–HPMC

Chitosan acetate (CSA) and HPMC can be applied at compression coat. Nunthanid et al.[140] prepared the tablet of 5-ASA using spray drying CSA and HPMC new compression-coats. They studied the effect of pH and enzymatic degradation of the tablet for suitability in colon delivery. The degradation of CSA by β-glucosidase in the colonic fluid enhanced the drug release while adding the disintegrant or the osmotic agent in the core tablets would affect the drug release.Priscileila et al.[141] prepared the enteric coated chitosan based drug delivery system for colon specific delivery of metronidazole. Enteric polymers, namely CAP and HPMCP, were incorporated, due to their insolubility in environments presenting low pH values. The results demonstrated that it is possible to prepare relative simple drug carrier systems able to reach the colonic environment, since their swelling capacity can be controlled by varying the composition.

3.1.10 Alginate–chitosan

Menniniet al.[142] designed a multiparticulate system, for colon-specific delivery of celecoxib for both systemic (in chronotherapic treatment of arthritis) and local (prophylaxis of colon carcinogenesis) therapy. The system comprised of ternary complexation with hydroxypropyl-β-cyclodextrin and PVP (polyvinylpyrrolidone), to increase drug solubility, and vectorization in chitosan-Ca-alginate microspheres, to exploit the colon-specific carrier properties of these polymers. Statistical experimental design was employed to investigate the combined effect of four formulation variables, i.e. % of alginate, $CaCl_2$, and chitosan and time of cross-linking on microsphere entrapment efficiency and drug released after 4 h in colonic medium. Design of experiment was used in the context of Quality by Design (QbD), which requires a multivariate approach for understanding the multifactorial relationships among formulation parameters. The desired goals were achieved for both systemic and local use of celecoxib. These results indicated that effectiveness of the proposed jointed use of drug cyclodextrin complexation and chitosan-Ca-alginate microsphere vectorization.

3.1.11 Ethyl cellulose–starch combination

Freire et al.[143] prepared the colon targeting pellets coated with a dispersion of high amylose starch (Hylon VII) and ethylcellulose (Surelease) (1:2, w/w) of (5-aminosalicylic acid; 5-ASA). Developed formulation was evaluated in vivo in rabbits. The coated tablets were able to resist the drug release in stomach and small intestine and were able to deliver maximum load to the colon. A novel polymeric film for the site-specific delivery of drugs to the colon of patients suffering from inflammatory bowel diseases was developed. Ethylcellulose (EC) was blended with different types of bacteria-sensitive starch derivatives. The water uptake and dry mass loss kinetics of the systems were monitored upon exposure to media simulating the contents of the stomach, small intestine and colon (including fresh fecal samples from Crohn's disease and Ulcerative Colitis patients). Importantly, EC: Nutriose FB 06 and EC: Peas starch N-735 films showed highly promising water uptake and dry mass loss kinetics in all the investigated media, indicating their potential to minimize premature drug release in the upper gastro-intestinal tract, and allowing for controlled release once the colon is reached[144,145].

Karrout et al.[145] studied the film coated pellets of Eurylon 6 HP-PG (a hydroxypropylated and pregelatinized high amylose starch) and ethyl cellulose for inflammatory bowel diseases. 5-ASA release could effectively be suppressed in 0.1 N HCl and phosphate buffer pH 6.8, optionally containing pepsin or pancreatin, but occurred as soon as the pellets came into contact with culture medium inoculated with fecal samples from inflammatory bowel disease patients. This can be attributed to the partial degradation of the starch derivative by enzymes secreted by bacteria present in the colon of patients. The developed formulation can be adapted to the pathophysiological conditions in inflammatory bowel disease patients. Furthermore, drug release remained unaltered upon 1 year open storage. Further this group studied the 5-ASA loaded beads prepared by extrusion–spheronization and coated with different Nutriose:EC blends. Interestingly, the release of 5-ASA could effectively be suppressed upon exposure to release media simulating the conditions in the upper GIT, irrespective of the degree of agitation and presence or absence of enzymes. But as soon as the pellets came into contact with fecal samples of inflammatory bowel disease patients, the release rate significantly increased and the drug was released in a time-controlled manner[146]

3.1.12 Ethyl cellulose–carbopol combination

Ali et al. prepared the matrix tablet of indomethacin for colon cancer, using different combination of ethyl cellulose and carbopol. The presence of ethyl cellulose in a hydrophilic polymer matrix resulted in a sigmoidal in vitro drug release pattern with negligible-to-very low drug release in the initial phase (0–6 h) followed by controlled release for 14–16 h. The decrease in initial release can be due to the ethyl cellulose that controls the swelling of hydrophilic polymer(s), while in the later portion, polymer relaxation at alkaline pH due to the ionization of acrylic acid units on carbopol and polycarbophil resulted in improved indomethacin release. A sigmoidal release pattern was obtained that could be ideal for colonic delivery of indomethacin in the potential treatment of colon cancer[147].

3.1.13 Pectin–chitosan

Bigucci et al. examined the release behaviors of vancomycin from poly electrolyte complex of pectin–chitosan. Moreover, the particular composition of these complexes improved vancomycin availability at alkaline pH on the bases of an enzyme-dependent degradation as confirmed from release studies performed in presence of beta-glucosidase[148]. Further this group extended the research and prepared hydrogels system of vancomycin using pectin and chitosan. Their study suggested that pectin/chitosan microspheres were able to limit the release of vancomycin under acidic conditions and release it under simulated colonic conditions, confirming their potential for a colon-specific drug delivery system[149].

3.1.14 Amidated pectin–chitosan–enteric polymers

Giselle et al.[150] prepared the multiparticulate colon specific delivery system of triamcinolone using chitosan and amidated pectin. HPMCP and CAP were successfully incorporated into the system and aided the target action of the carbohydrates. The in vitro drug release studies showed that the addition of both enteric polymers, CAP and HPMCP, to the PC:CS:TC particles resulted in higher control over drug release in all media analyzed. Particles from all charges also exhibited enzyme-controlled drug release properties in simulated colonic medium. The addition of CAP and HPMCP resulted in the highest control over the drug release in all media. CAP:TC formulation presented the slowest drug release rate, of only 1.33%, in acidic medium after 2 h, while the control formulation released 45.52% after the same time.

3.1.15 Guar gum–chitosan

Celecoxib loaded polysaccharide films of guar gum and chitosan for local adjuvant or neoadjuvant therapy of colorectal cancer was developed. The impact of a single high dose was evaluated and compared with a repeating low-dose regimen. In vivo dosing experiments with Cx were performed in the perfused intestine of the anaesthetized rat. The study suggested maximum therapeutic efficiency in the context of minimal healthy tissue exposure for utilizing a local delivery system such as the proposed adhesive, biodegradable polysaccharide composites[151]. Ravi et al.[152] prepared novel colon targeted tablet formulation using natural polysaccharides such as chitosan and guar gum as carriers and diltiazem hydrochloride as model drug. In vitro studies revealed that the tablets coated with inulin and shellac have controlled the drug release in stomach and small intestinal region and released maximum amount of drug in the colonic environment. The study revealed that polysaccharides as carriers and inulin and shellac as coating materials can be used effectively for colon targeting of drugs for treating local as well as systemic disorders.

3.1.16 Guar gum–alginate

A matrix tablet of guar gum and alginate for colon specific delivery of ondasatron for the treatment of IBD was prepared by Tugcu. The developed formulation was system able to reduce the visceral sensitivity and inhibition of motor activity in irritable bowel syndrome (IBS) [153].

3.1.17 Chitosan–alginate

Lectin-conjugated chitosan-Ca-alginate microparticles (MPs) loaded with acid-resistant particles of 5-fluorouracil (5-FU) for efficient local treatment of colon cancer were prepared by Glavas et al.[154].Microparticles were prepared by a novel one-step spray-drying technique and after wheat germ agglutinin (WGA) conjugation. The retention of biorecognitive activity of WGA after covalent coupling to MPs was confirmed by haemagglutination test. Functionalized MPs showed excessive mucoadhesiveness in vitro, due to the positive surface charge, pH-dependent swelling of the matrix and lectin–sugar recognition. Laroui et al.[155] studied nanoparticles (NPs) to deliver an anti-inflammatory tripeptide Lys-Pro-Val (KPV) to the colon and assessed its therapeutic efficacy in a mouse model of colitis. To target KPV to the colon, loaded NPs (NP-KPV) were encapsulated into a polysaccharide gel containing 2 polymers: alginate and chitosan. The effect of KPV-loaded NPs on inflammatory parameters was determined in vitro as well as in the dextran sodium sulfate-induced colitis mouse model. The studied suggested that by using NPs, KPV can be delivered at a concentration that is 12,000-fold lower than that of KPV in free solution, but with similar therapeutic efficacy. Administration of encapsulated drug-loaded NPs can be a novel therapeutic approach for IBD.

3.1.18 Dextran–chitosan

A polyelectrolyte complex (PEC) consisting porous chitosan (CS) hydrogel microsphere of ibuprofen were prepared via either wet phase-inversion or ionotropic crosslinking with sodium tripolyphosphate (Na^+-TPP) and dextran sulfate (DS). The CS/TPP/DS microspheres resisted hydrolysis in strong acid and biodegradation in enzymatic environments. The swelling kinetics for CS microspheres was close to Fickian diffusion, whereas those for CS/TPP and CS/TPP/DS were non-Fickian. The release profiles of ibuprofen from CS/TPP/DS microspheres were slow in simulated gastric fluid (SGF, pH 1.4) over 3 h, but nearly all of the initial ibuprofen content was released in simulated intestinal fluid (SIF, pH 6.8) within 6 h after changing media. Overall the results indicated that CS/TPP/DS microspheres could successfully deliver a hydrophobic drug to the intestine without drug degradation in the stomach, and hence could be potential candidates as an orally administered colon drug delivery system[156].

3.1.19 Pectin–ethylcellulose

The first studies have been conducted with pectin/ethylcellulose aqueous dispersion mixtures (Surelease® — formulations containing 40–60% pectins) to coat paracetamol pellets. With this type of coating, 5–30% of the paracetamol was released within 6 h at pH 7.4 in a dissolution medium without enzymes. In the presence of pectinolytic enzymes, the release of the drug increased and this was even more marked when the coatings contained high proportions of pectins[157]. In vitro trials have confirmed that increasing the coating layer reduced drug release[157]. Thus, ethylcellulose reduces the hydrosolubility of a coating containing pectins and increases the impermeability of the coating. Stability studies have shown that coalescence of the film coating no longer occurred following the coating process. The techniques used to apply the film coating to the dosage forms on the one hand affect the release kinetics of the drug and, on the other, the stability of this type of dosage form[158-160].

Finally, the high methyl ester pectin concentrations in coatings that also contain Aquacoat® ECD 30 must be between 0 and 20% (w/w) to give the film coatings certain interesting mechanical properties[161-163]. On the basis of previously obtained results, studies have been conducted with high methyl ester pectins and calcium pectinate combined with Aquacoat® ECD 30 or Surelease®. These film coatings contained 5, 10 and 15% (w/w) of pectins or 10% (w/w) of calcium pectinate and dibutyl sebacate and triacetine as plasticizers. The film coatings containing high methyl ester pectins and Aquacoat® ECD 30 released at least 80% of the pectins within 1 h in a dissolution medium without pectinolytic enzymes at pH 7.5, whatever the pectin concentration and irrespective of the presence or absence of calcium ions. On the basis of the results obtained with these film-coating compositions, it was concluded that these were not suitable for targeting drugs to the colon.

The nature of the ethylcellulose aqueous dispersion also affects the release kinetics of pectins [164,44]. For film coatings containing Surelease®, pectin release in a dissolution medium without pectinolytic enzymes depends on the high methyl ester pectin concentration. Film coatings containing 5% pectins released about 50% of the total pectin quantity after 4 h at pH 4.5. Film coatings containing 10% pectins released 80% of the total quantity after 1 h. have also suggested that the pectin concentration released by a film-coating composition containing 5 and 10% probably depends on the porosity of the film coating, which is lower in a composition containing only 5%[164,44].

3.2 Carbohydrate–Eudragit Mixtures

The carbohydrate–Eudragit mixtures can be a better promising approach for colon delivery. Eudragit series is available with different form. The different Eudragit polymers have the property of dissolving at specific pH value. Different combinations of carbohydrate polymers with Eudragit are described in this section.

3.2.1 Guar gum–Eudragit

Ji et al.[165] prepared pH and enzyme-dependent colon-targeted multi-unit delivery system of indomethacin by coating guar gum and Eudragit FS30D sequentially onto drug-loaded pellets in a fluidized bed coater. Pharmacokinetic study in beagle dogs showed that fastest absorption with the smallest T_{max} and T_{lag} was observed for uncoated pellets. The T_{max} and T_{lag} of Eudragit FS30D-coated pellets were postponed to about 2.5 and 1 h, respectively. After a further guar gum coating, T_{lag} was further postponed to about 2.8 h, about 2 h of additional lag time on the basis of Eudragit FS30D coating. The results indicated that the guar gum/Eudragit FS30D-coated system has potential to be used to deliver drugs to the colon. The chronotherapeutic based colon-targeted drug delivery system of theophylline (THEO) exploiting pH-enzyme sensitive property was prepared the prevention of episodic attack of asthma in early morning. Guar gum microspheres of theophylline were prepared by emulsification technique. Coating of microspheres was performed using solvent evaporation method with pH sensitive Eudragit® polymers. The controlled release of THEO after a lag time was achieved with developed formulation for chronotherapeutic delivery. The pH dependent solubility behavior of Eudragit and gelling properties of guar gum are found to be responsible for delaying the release[166].

Ji et al.[167] prepared colon specific guar gum-based multi-unit pellet system. The guar gum was coated by pH-sensitive polymer Eudragit FS30D sequentially around drug-loaded non-pareil cores in a fluid-bed coater. The outer Eudragit FS coating protects the system against gastrointestinal environment and dissolves rapidly in distal small intestine, where a lumen pH of over 7 triggers the dissolution of the enteric polymer. The inner guar gum coating works as a time-controlled retardant and offers additional protection of the pellets until it is degraded by microbial enzymes at the proximal colon. In vitro results indicate that guar gum is a feasible coating material to achieve timed and enzyme-triggered fluorouracil release. Pharmacokinetic study in beagle dogs shows delayed absorption of about 5 h and limited absorption fraction as a result of guar gum and Eudragit FS coating.

3.2.2 Pectin–Eudragit

The Eudragit S-100 based microsponges bearing dicyclomine for colonic delivery was developed. The colon-specific tablets were prepared by compressing the microsponges followed by coating with pectin: HPMC mixture. In vitro release studies exhibited that compression-coated colon-specific formulations started releasing the drug at the sixth hour corresponding to the arrival time at colon. The study presented a new approach for colon-specific drug delivery[168]. Matrix systems containing calcium pectinate (mixture of calcium pectinate/pectins and calcium pectinate/guar gum) have been evaluated *in vivo*[169]. The resulting tablets have been coated with an enteric acrylic polymer such as Eudragit® L. The disintegration of all the tablets started in the last part of the ileum and continued in the colon. These dosage forms were not suitable for the specific release of hydrophilic drugs in the colon, since they are released before reaching the colon.

In the absence of pectinolytic enzymes, a reduction in the release kinetics of pectins from film coatings made of Eudragit® NE 30 D and 10% calcium pectinate was observed. Comparison of the same film coatings containing 5–10% (w/w) low methyl ester or high methyl ester pectins without calcium made it possible to conclude that this can be attributed to the presence of calcium (30–60 mg Ca/g of pectin). The release delay depends on the calcium/pectin ratio and appeared to be optimal when Eudragit® NE 30D is combined with calcium pectinate at a concentration of 30 mg Ca/g of pectins. Macromolecules of pectins leave the polymer coating via a diffusion mechanism and create pores. Similarly, drugs of low molecular weight are known to diffuse through Aquacoat® ECD 30, Eudragit® RS 30D and RL 30D polymeric film coatings.

With respect to films containing Eudragit® RS, pectin release was slow and remained constant for about 8 h (~10% of pectins released) for films containing 5 and 10% high methyl ester pectins and 10% low methyl ester pectins. Release was more rapid when the film coatings contained 15% high methyl ester pectins (40% of pectins released after 8 h) and almost complete in less than 1 h with calcium pectinate (low methyl ester pectins+calcium). These results can be attributed to the fact that the quaternary ammonium groups are able to interact with carboxylic groups of high methyl ester pectins to form a pectin–Eudragit® RS complex that prevents the release of pectins. The existence of these interactions is also demonstrated with a film coating composed of a mixture of calcium pectinate and Eudragit® RS. In this case, almost all the pectins were released in under an hour. The addition of 30 mg Ca/g to low methyl ester pectins seemed sufficient to prevent any interaction between the pectins and Eudragit® RS 30D. As far as pectin release is concerned, film coatings made of Eudragit® RS 30D and calcium pectinate with a ratio of 30 mg Ca/g of pectins demonstrated similar performances as those of film coatings composed of high methyl ester pectins or calcium pectinate with Aquacoat® ECD 30, Surelease® or Eudragit® NE 30D. The release kinetics of pectin from isolated film coatings showed that only combinations of Eudragit® RS 30D with high methyl ester pectins or low methyl ester pectins (10% (w/w)) were able to possess the two essential properties (gastrointestinal resistance and degradation/dissolution in the colon) to guarantee specific drug delivery to the colon.

Study of the release kinetics of pectins from film coatings containing Eudragit® RS 30D in a buffer solution containing pectinolytic enzymes has shown that the release kinetics of pectins and their release-rate are governed by the pectin content of the film coatings. Film coatings containing 5% high methyl ester pectins are not permeable enough to the pectinolytic enzymes, leading to a significant reduction in pectin release from film coatings incubated with and without pectinolytic enzymes. Combination of high methyl ester pectins or calcium pectinate with insoluble polymers did not lead to specific drug release in the colon. However, a pectin that is an anionic polymer is able to form an insoluble complex with a cationic polymer such as Eudragit® RL, which is not degraded by the enzymes of the colonic flora. The best solute release kinetics in the presence or in the absence of enzymes are obtained for film coatings composed of 10–15% (w/w) pectins and Eudragit® RL. The incorporation of a sufficiently high ratio of this type of complex in film coatings that also contain insoluble and flexible polymers such as Eudragit® NE 30 D makes it possible to obtain an increase in drug release following the action of pectinolytic enzymes. Indeed, since the pectin–Eudragit® RL complex incorporated is insoluble, the film coating will be less permeable to the drug in the absence of pectinolytic enzymes.

Enzymatic degradation and the consecutive release of pectin from the film coating will lead to the destruction of the complex, allowing the Eudragit® RL to regain its original physicochemical properties. The polymer will then be able to solvate, to swell and to increase release of the drug[44]. With respect to isolated films made of either pectins+Eudragit® RS 30D or pectins+Eudragit® NE 30D, the action of pectinolytic enzymes made it possible to decrease the release kinetics of theophylline through the isolated films compared with the release kinetics without enzymes. Indeed, spaces created by enzymatic degradation are filled due to reorganisation of the polymer macromolecules[45].

3.2.3 CAP–Eudragit

Kotagale et al.[170] prepared polymer-coated polysaccharide tablets for colon specific delivery of azathioprine. Tablets were prepared by direct compression method using different ratios of avicel-Micro Crystalline Cellulose (MCC), inulin and triacetin. Eudragit-S, Eudragit-L and cellulose acetate phthalate (ES, EL and CAP) were used for coating. Drug release increased with the plasticizer (triacetin) concentration. Increase in the concentration of inulin and citric acid above 5% (w/w) increases the drug release. The addition of inulin in the formulation with coating level 28% (w/w) demonstrated increased drug release in presence of rat cecal content. The results revealed that inulin containing ES, EL and CAP (1:1:1) polymer-coated formulation system can be used for the targeted delivery of azathioprine with desired release pattern.

3.2.4 Chitosan–Eudragit

Hyaluronic acid-coupled chitosan nanoparticles bearing oxaliplatin (L-OHP) encapsulated in Eudragit S100-coated pellets were developed for effective delivery to colon tumors. In murine models, the drug delivery system showed relatively high local drug concentration in colonic tumors with prolonged exposure time, which provides a potential for enhanced antitumor efficacy with low systematic toxicity[168, 171].

Ibekwe and co-workers reported a novel dual-triggered colonic delivery system with improved site-specificity over the pH-responsive systems currently used for ulcerative colitis. The system consisted of a mixture of pH-responsive Eudragit S and resistant starch in a single layer matrix film. Tablets were administered in a three-way crossover study to eight healthy volunteers. The site of intestinal disintegration was assessed using gamma scintigraphy. The coated tablets were able to resist breakdown in the stomach and small intestine. The dual pH- and bacterially-triggered coating was applied to tablets and dosed a total of 23 times in healthy volunteers under three different feeding conditions (i) without food, (ii) with breakfast or (iii) 30 min before breakfast. The tablet did not empty from the stomach during the study period, but of those 22 tablets that emptied, disintegration occurred at the ileo-caecal junction or in the large intestine confirming successful targeting with this system. The independent triggers of a bacterially-triggered component within a pH-responsive polymer are effective, complementary and act as failsafe mechanisms for each other[172].The Eudragit-S-100 coated chitosan microspheres for 5-ASA and camylofine dihydrochloride for the treatment of ulcerative colitis was prepared by Dubey et al. In vivo data showed that microspheres delivered most of its drug load (76.55 ± 2.13%) to the colon after 9 h, which reflects its targeting potential to the colon. The study suggested that orally administered microspheres of both drugs can be used together for the specific delivery of drug to the colon and reduce symptoms of ulcerative colitis[173, 174].

3.2.5 Inulin–Eudragit RS

Coatings made of inulin with a mean polymerization degree of 24 mixed with Eudragit® RS are resistant to gastric and intestinal juices and are effectively degraded by the colonic flora. Inulin can therefore be used to lead to specific release of drugs in the colon. The bacterial degradation of films led to a fall in pH in the micro-environment due to the generation of lactic and acetic acids and other volatile fatty acids[175]. The plasticizers used to make these coatings are dibutylphthalate and acetyltriethylcitrate at a concentration of 20% (w/w) of the dry mixture. The hydrophilic character of the plasticizer increases the accessibility of the inulin for the colonic flora. Improvements still need to be made to this composition for better control of drug release at this site.

4. CHEMICAL APPROACHES FOR BACTERIALLY TRIGGERED SYSTEM

Polysaccharides are used as glucuronic prodrugs, which are specifically degraded by colonic β-glucuronidases and glycosidic prodrugs, which are specifically degraded by colonic glycosidases [13, 14]. The most widely used polysaccharide of this type is dextran. The action of bacterial glycosidase enzymes on the glycosidic bond permits the release of the attached drug, then triggering its pharmacological activity[40]. Biolabile prodrug compounds are prepared from a polysaccharide with a molecular weight (M_w) ranging from 40,000 up to 5,000,000, selected from dextran, carboxymethyl dextran, diethylaminoethyl dextran, starch, hydroxyethyl starch, alginates, glycogen, pullullan, agarose, cellulose, chitosan, chitin and carrageenan. Following the oral administration of these prodrugs, the parent drug is selectively released in the terminal ileum and the colon over an extended period of time[68]. Using β-cyclodextrins as carriers for 5-amino salicylic acid (5-ASA) in the form of prodrugs makes it possible to prevent the release of more than 2% of 5-ASA in simulated gastric

and intestinal fluids. The active ingredient is released, in particular, by degradation of the prodrug by the colonic microflora. For oligopeptides, this approach can only be investigated when they are not degraded by intestinal bacteria. High levels of short chain fatty acids (SCFA), such as acetate, propionate and butyrate, can be targeted to have beneficial effects in the prevention of colonic disorders (rectal cancer, diverticulitis, colitis, diarrhoea and constipation) by using an ester covalently linking the SCFA to a carrier that is preferably a form of carbohydrate. The SCFA were protected by their link with the carbohydrate as they passed through the small intestine. The carbohydrates chosen for this application were digestible in the small intestine, such as digestible starch, which can also be protected from digestion in the small intestine by substitution.

4.1 Glycoside conjugates

Steroid glycosides and the unique glycosidase activity of the colonic microflora form the basis of a new colon targeted drug delivery system. Drug glycosides are hydrophilic and thus, poorly absorbed from the small intestine. Once such a glycoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to be absorbed by the colonic mucosa. The major glycosidases identified in human feces are b -D-galactosidase, b -D-glucosidase, a -L-arabinofuranosidase, b -D-xylopyranosidase.[101]These enzymes are located at the brush border and hence access to the substrate is relatively easy. In the plant kingdom numerous compounds are found as glycosides. Certain drugs act as glycon and can be conjugated to different sugar moieties which results in the formation of glycosides. Due to the bulky and hydrophilic nature of these glycosides, they do not penetrate the biological membrane upon ingestion[12].Various naturally occurring glycosides, e.g. the sennosides, have been used for laxative action for ages. When taken orally, intact sennosides are more efficient as laxative than sugar free aglycones. These sennosides are activated by colonic microflora to generate rhein anthones, which gives the desired laxative effect[11]. Glycosidase activity of the GIT is derived from anaerobic microflora in the large bowel or the sloughed or exfoliated cells of the small intestine.Friend and Chang[13] prepared dexamethasone-21- b -glucoside and prednisolone-21- b -glucoside for delivery of these steroids to the colon.Hydrolysis of prodrugs by b -glucosidase and fecal homogenates *in vitro* released the free steroids. Glucosides were administered to rats intragastrically to determine when and where the free steroids were released. Unmodified dexamethasone and prednisolone were also given to rats intragastrically to compare absorption of the glucosides with the free steroids. Both glucosides were found to reach the rat lower intestine in 4-5 h, where they were rapidly hydrolyzed, releasing the free steroids. *In vivo* studies on dexamethasone- b -D-glucoside revealed that nearly 60% of an oral dose of glucoside reached the caecum whereas in case of prednisolone- b -D-glucoside, only 15% reached to the caecum. When free steroids were administered orally, they were almost absorbed in the small intestine and less than 1% of oral dose reached at the colon. The influence of prodrug structure on specificity of glycoside/glycosidase based colon-specific drug delivery was studied by preparing nine steroid glycosides, measuring their relative lipophilicities and hydrolyzing them with bacterial glycosidases from rat intestines[14]. The 21-yl b -D-glucosides and galactosides of dexamethasone, prednisolone, hydrocortisone and fludrocortisone and 21-yl b -D-cellobioside of prednisolone were prepared by a modified Koenigs-Knorr reaction. The deacetylated glycoside prodrugs along with the p-nitrophenyl derivatives of b -D-glucoside, galactoside and cellobioside were subjected to hydrolysis by the contents of the rat stomach, proximal small intestine (PSI), distal small intestine (DSI) and caecum. All the prodrugs were hydrolyzed slowly by PSI and stomach contents, more rapidly by contents of the DSI, and most rapidly by caecal contents. *In vitro* studies performed specifically on dexamethason- b -D-glucoside revealed that both GIT tissues and GIT contents of guinea pig showed b -glucosidase activity[15]. Among the tissues maximum activity was seen in tissues of PSI whereas among the contents maximum activity was seen in the caecum and the colon. For *in vivo* studies experimental IBD was induced using degraded carrageenan in guinea pig. 0.65 mol/kg dexamethason- b -D-glucoside was equally effective as 1.3 mol/kg of dexamethason alone in reducing the total number of ulcers. The results indicated that a lower dose of dexamethasone, administered, as its glucoside prodrug could be equally efficacious relative to higher dose of dexamethasone.

4.2 Cyclodextrin conjugates

Cyclodextrins (CyDs) are cyclic oligosaccharides consisted of six to eight glucose units through a -1, 4 glucosidic bonds and have been utilized to improve certain properties of drugs such as solubility, stability and bioavailability. The interior of these molecules is relatively lipophilic and the exterior relatively hydrophilic, they tend to form inclusion complexes with various drug molecules[17] .They are known to be barely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however, they are fermented by colonic microflora into small saccharides and thus absorbed in the large intestineBecause of their bioadaptability and multi-functional characteristics, CyDs are capable of alleviating the undesirable properties of drug molecules in various routes of administration through the formation of inclusion complexes. In an oral drug delivery system, the hydrophilic and ionizable CyDs can serve as potent drug carriers in the immediate release and delayed release-formulations, respectively, while hydrophobic CyDs can retard the release rate of watersoluble drugs. It has been proved through a

study in healthy human volunteers that b CyDs are meagrely digested in small intestine but are completely degraded by the microflora of the colon. Most bacterial strains that are isolated from human being are capable of degrading CyDs. It has been proved by their ability to grow on cyclodextrins by utilizing them as the sole carbon source and by the stimulation of cyclodextrinase activity by as low as 2-4 h of exposure to cyclodextrins. This property of the drug may be exploited for the formation of colon targeted drug delivery systems. An anti-inflammatory drug biphenylacetic acid (BPAA) as model drug was selectively conjugated onto one of the primary hydroxyl groups of a -, b - and γ- CyDs through an ester or amide linkage, and the *in vivo* drug release behavior of these prodrugs in rat gastrointestinal tract after oral administration was investigated. The CyD prodrugs were stable in rat stomach and small intestine and negligibly absorbed from these tracts. Three to six h after oral administration, most of the prodrugs had moved to the caecum and colon. The a- and γ-CyD amide prodrugs were hydrolyzed to the maltose conjugate in the caecum and colon, and these prodrugs and the conjugates were negligibly absorbed. On the other hand,the a-and γ-CyD ester prodrugs produced BPAA in the caecum and colon, and BPAA appeared in the blood after 3-6 h. Both b-CyD amide and ester prodrugs released only small or negligible amounts of the maltose conjugate or BPAA in the caecum and colon within 24 h, probably due to the low solubility in the biological media. Further, the anti-inflammatory effect of the γ-CyD ester prodrug was evaluated using the model of carageenan-induced acute edema in rat paw and compared with those of BPAA alone and the BPAA/b-CyD complex prepared by the kneading method in a molar ratio of 1:1. In the case of b-CyD complex, a rapid anti-inflammatory response was observed from the small intestine after a fast dissolution of the complex. In sharp contrast, the γ-CyD ester prodrug required a fairly long lag time to exhibit the drug activity, because BPAA was produced after the prodrug had reached the caecum and colon. These results clearly suggest that the CyD prodrug approach could provide a versatile means for constructions of not only colon-specific delivery systems but also delayed-release system of certain drugs[102, 103]. Hiramaya et al.[17] prepared two CyD conjugates where one primary hydroxyl group of b-CyDs was substituted by BPAA through an ester or amide linkage. Aqueous solubility of the conjugates was lower than those of other drug or parent compound. The amide conjugate was stable in aqueous solution and in rat biological fluids and gastrointestinal contents. The ester conjugate released the drug preferentially when incubated with the contents of caecum or colon, whereas no appreciable drug release was observed on incubation with the contents of either stomach or intestine in intestinal or liver homogenates or in rat blood. Prednisolone, a typical glucocorticoid, has been widely used for the treatment of IBD. However, when Prednisolone is administered orally, a large amount of the drug is absorbed from the upper GIT and causes systemic side effects. The anti-inflammatory effect and systemic side effect of the prednisolone succinate/alpha-cyclodextrin ester conjugate after oral administration were studied using IBD model rats. The systemic side effect of the conjugate was much lower than that of prednisolone alone when administered orally. The lower side effect of the conjugate was attributable to passage of the conjugate through the stomach and small intestine without significant degradation or absorption, followed by the degradation of the conjugate site-specifically in the large intestine[104].

4.3 Dextran conjugates

Dextran ester prodrug was prepared and *in vitro* release revealed that release of naproxan from prodrug was several folds higher in caecum homogenates than in control medium orhomogenates of the small intestine of pig[16].The bioavailability of naproxan after oral administration of a dextran T-70-naproxan ester prodrug in pigs was assessed by Harboe et al.[16] Compared to the administration of an oral solution of an equivalent dose of naproxan the average absorption fraction for the conjugate amounted to 91%. It was established that several features of the prodrug indicated that naproxan was released from the prodrug prior to systemic absorption and that drug activation involved the action of one or more enzyme systems located in the gastrointestinal tract. It was observed in rabbits, the plasma concentration-time curves for the conjugate were characterized by an initial lag time of about 2-3 h, whereas naproxan was detected in plasma immediately after per oral administration of the drug compound per se. The distribution of the prodrug along the GIT at various times after conjugate administration was assessed qualitatively by HPLC analysis of conjugated and free naproxan in various segments of the GIT. From these experiments it was suggested that drug regeneration was effective in the bowel below the ileum[68,69]. Dextran ester prodrugs of metronidazole have been prepared and characterized[70].Mcleod et al.[71] synthesized dextran ester prodrugs of dexamethasone and methylprednisolone and proved the efficacy of the prodrugs for delivering drugs to the colon. In this study, methyl prednisolone and dexamethasone were covalently attached to dextran by the use of a succinate linker. In addition, dexamethasone was attached by glutaric acid to investigate the effect of linker molecule on hydrolysis kinetics. The kinetics of degradation of the hemiester and corresponding dextran conjugates were measured as a function of pH and temperature. Intermolecular migration of the linker molecule from the 21- to the 17-position on the glucocorticoid occurred in all three hemiester, although to a greater extent in methylprednisolone-hemiester. The dextran conjugates were also incubated at 37°C; pH 6.8 and the chemical degradation half-lives were determined. Dexamthasone-21-hemisuccinate showed half life of 75 h, dexamethasone-glutaratedextran exhibited half life of 103 h, while methylprednisolone-succinate-dextran showed half life of 82 h.

Glucocorticoids remain the foundation of therapy for acute ulcerative colitis despite systemic side effects that limit their use. Prodrugs that selectively deliver glucocorticoids to the colon may lower the required dose and side effects.

5. POLYSACCHARIDE BASED BACTERIALLY TRIGGERED SYSTEMS/TECHNOLOGY

5.1 COLAL-PRED Technology

COLAL-PRED® technology was developed by Alizyme Therapeutics Limited. COLAL-PRED® comprises small pellets containing prednisolone metasulfobenzoate sodium (PMSBS) with a coating of ethyl cellulose and a specific form of amylose (derived from starch) that is broken down only in the colon by enzymes from locally occurring bacteria. This enables PMSBS to be taken orally and delivered topically to the colon, rather than systemic delivery, since release in the stomach and small intestine is prevented. This makes possible the effective treatment of ulcerative colitis without the usual debilitating side effects typically associated with such steroids. It has been shown in a Phase III clinical trial to provide a significantly improved risk-benefit profile to that of conventional oral prednisolone.

5.2 ENCODE-Phloral™

ENCODE technology was developed by ENCAP drug delivery, includes the Phloral™ system licensed from the School of Pharmacy in London. This is a unique coating technology that is designed to target the release of drugs to the colon region. It consists of a blend of bacteria-activated (starch) and pH-activated (Eudragit S) component. The dual pH- and bacterially-triggered coating was applied to tablets and dosed a total of 23 times in healthy volunteers under three different feeding conditions. On one occasion, the tablet did not empty from the stomach during the study period, but of those 22 tablets that emptied, disintegration occurred at the ileo-caecal junction or in the large intestine confirming successful targeting with this system.

The independent triggers of a bacterially-triggered component within a pH-responsive polymer are effective, complementary and act as failsafe mechanisms for each other. This platform technology could theoretically be adapted to any drug and used for a variety of disease states. The clinical potential for inflammatory bowel disease is obvious with a low risk of dose dumping and low risk of the tablets passing intact and for new systemic applications for colonic delivery[154].

5.3 Clipper

Clipper® is gastro-resistant prolonged-release tablet of beclometasone dipropionate. This technology was developed by Chiesi Limited, UK. The tablet core consists of hypromellose (E. 464), microcrystalline cellulose maize starch, etc., while the tablet coating was made up of Eudragit L100-55. The tablets are indicated for the treatment of mild or moderate ulcerative colitis in active phase, as add-on therapy to 5-ASA containing drugs in patients who are non-responders to 5-ASA therapy in active phase[176].

5.4 GLARS (geometrically long absorption regulated system)

The focus of GL Pharm Tech over the past ten years has been on developing a technology named GLARS. The system entraps more gastro-intestinal fluid into the dosage form at early dissolution time to give further extended absorption in the colon. GLARS technology developed by GL Pharm Tech (Korea) consists of oral sustained-release triple layer tablet containing (1) an inner immediate-release layer comprising a therapeutically active ingredient and (2) two outer layers having swellable polymers, wherein, upon exposure to aqueous media, the exposed lateral side of the inner immediate-release layer is surrounded by the two swollen outer layers to control the release of active ingredient. The swellable polymers are selected from cellulose derivative. The technology was evaluated for its proof of concept on tianeptine and tamsulosin[177].

5.5 Chrono Cap

Chrono Cap is patented technology of Universita degli Studi di Milano-IT. The Chrono Cap technology relates to an oral capsular device intended for pulsatile (time-controlled) release of drugs to colonic region. The Chrono Cap can optionally be coated with gastric-resistant polymers (HEC, HPMC, PVP, HPC, etc.) thus being adapted to a time-dependent colon delivery system. Such capsules are prepared from hydrophilic polymeric materials that undergo a glassy–rubbery transition when exposed to aqueous fluids, thereby delaying the release of the contents for a programmable period of time following administration. The lag time that precedes the onset of release can be modulated as a function of the thickness and composition of the capsule shell. Chrono Cap devices can be filled just like hard gelatin capsules and may convey solid (powders, capsules, tablets, granulates, pellets, micro- or nano-particles), semi-solid or liquid drug formulations. Colonic release is of high interest not only for the therapy and prevention of pathologies that affect the large intestine (ulcerative colitis, Crohn's disease, colorectal adenocarcinoma, microflora disorders), but also for pharmacological treatments that require a systemic absorption of the drug[178-182].

Table 1: Chemically modified carbohydrate polymers in colon specific drug delivery application[112-116]

Polymers used	Modification in polymer	Drug delivery system prepared	Bioactive studied	Purpose/outcome of study
Guar gum	Acetyl derivative; O-acetyl-galactoglucomannan was prepared	Hydrogel	BSA	Modified AcGGM decreased the hydrolysis rate and maximum hydrolysis, indicating steric hindrance of the enzyme by the acrylate side group.
Arabinogalactan	Tethering with folic acid	Biomacromolecular nanovehicle	Methotrexate	The FA-AG-GFLG-MTX drug conjugate displayed 6.3-fold increased cytotoxic activity to FR-overexpressing cells compared to their FR-lacking counterparts.
Pectin	Oxidized citrus pectin	In situ hydrogels	Doxorubicin	Oxidized pectin hydrogels have the potential to prevent both progression of primary cancer by the released Doxorubicin and generation of metastatic cancer by the released Oxidized pectin.
Chitosan	Folate complex prepered	Microcapsule	Camptothecin	The chitosan-folate microcapsules loaded with camptothecin significantly reduced the proliferation of HeLa tumor cells, while they have a negligible effect on fibroblasts.
Dextran	Methacrylated and succinic derivative prepared	Hydrogel	2-Methoxyestradiol (Model drug)	*In vitro* cell compatibility studies indicated the absence of toxic effects. Potential mucoadhesive behavior of the hydrogel promoted drug release in the site of action for a prolonged time.

6. CONCLUSION

There is an increasing interest in targeted delivery of drug to the colon via the oral route. Targeting drugs to the colon has major advantages in the direct treatment of the local disease and also for allowing the possibility of using colon for systemic therapy since the residence time is more than 24 h. The vagaries in pH of different organs of the GIT pose problems for those systems that take into consideration specific values of pH for their activation. Microflora-activated systems appear to be more promising because the abrupt increase of the bacteria population and associated enzyme activity in the colon represent a non-continuous event independent of gastrointestinal transit time.Chemical modifications made to polysaccharides make it possible to reduce release of the drugs in the gut. However, the kinetics of degradation and of solute release from hydrogels depends on numerous parameters and on the nature of the drugs.Combinations of polysaccharides and polymers that are either insoluble or soluble at colonic pH have been tested. These combinations are based on the erosion and swelling of film coatings all along the gastrointestinal tract and degradation of polysaccharides in the colon.Polysaccharides with a large number of derivatizable groups, a wide range of molecular weight, varying chemical composition and above all being stable, safe and biodegradable, offer properties preferable over all the other approaches. A number of studies have been conducted on plain and derivatized pectin, guar gum, dextran and chitosan. Relatively lesser amount of studies have been conducted on chondroitin, insulin, alginates, amylose etc. However, a substantial amount of research remains to be conducted to develop a polysaccharide based colon-specific drug delivery dosage form which is easier and simpler to formulate and is highly site-specific.

REFERENCES

1. Sarasija S, Hota A. Colon specific drug deliverysystems. Indian J Pharm Sci 2000; 62:1-6.
2. Saffran M, Bedra C, Kumar GS, Neckers DC.Vasopressin: a model for the study of effects ofadditives on the oral and rectal administration ofpeptide drugs. J Pharm Sci 1988; 77(1):33-8.
3. Mackay M, Tomlinson E. Colonic delivery oftherapeutic peptides and proteins In:Colonic drugabsorption and metabolism. MarcelDekker, NewYork, 1993; 159-176.
4. Lee V HL. Changing needs in drug delivery .In: Peptide and protein drug delivery. Marcel Dekker, New York, 1.
5. Ikesue K, Kopeckova P, Kopecek J. Degradationof Proteins by Enzymes of theGastrointestinalTract.Proc Int Symp Control RelBioact.mater1991; 18:580-581.
6. Digenis GA, Sandefer E. Gamma scintigraphy andneutron activation techniques in the *in vivo* assessment of orally administered dosage forms. CritRev Ther Drug Carrier Syst. 1991; 7(4):309-45.
7. Taniguchi K, Muranishi S, Sezaki H. Enhancedintestinal permeability to macromolecules. II.Improvement of the large intestinal absorption ofheparin bylipid-surfactant mixed micelles in rats. IntJ Pharm 1980; 4:219-228.
8. Hardy JG. Colonic transit and drug delivery."In:drug delivery to the gastrointestinal Tract. 1989:75.
9. Jay M, Beihn RM, Digenis GA, Deland FH,Caldwell L, Mlodozeniec AR. Disposition of radiolabelled suppositories in humans. J PharmPharmacol 1985; 37(4):266-8.
10. Wood E, Wilson CG, Hardy JG. The spreadingoffoam and solution enemas. Int J Pharm1985; 25:191-197.
11. RileySA.TurnbergSulphasalazine and the aminosalicylates in the treatment of inflammatory bowel disease. J Med 1990;75 : 551–562
12. Khan KA, Piris J, Truelone SC. An experiment to determine the active therapeutic moiety of sulphasalazine. Lancet1977; 2: 895-898.
13. FriendDR, Chang GW. A colon-specific drug delivery system based on drug glycosides and glycosidase of colonic bacteria. J Med. Chem 1984 ;27: 261–266.
14. FriendDR, Chang GW. Drug glycosides: potential prodrugs for colon specific drug delivery. J Med Chem 1985; 28:51–57.
15. FriendDR, Tozer TN. Drug glycosides in oral colon-specific drug delivery. J Control Release.1992;19: 109–120.
16. Harboe E, Larsen C, Johansen M, Olesen HP.Macromolecular prodrugs XIV. Absorption characteristics of naproxen after oral administration of a dextran T-70-naproxen ester prodrug in pigsInt. J Pharm 1989; 53:157–165.
17. Hirayama F, Minami K, Uekama K. In vitro evaluation of biphenylyl acetic acid-β-cyclodextrin conjugates as colon targeting prodrugs: drug release behaviour in rat biological media. J Pharm Pharmacol 1996; 48: 27–31.

18. Davis JG, Hardy M J, Taylor, Stockwel A, Whalley DR, Wilson CG. The in vivo evaluation of an osmotic device (osmet) using gamma scintigraphy. J Pharm Pharmacol 1984; 36: 740–742.
19. Wilson CG, Washington N. Physiological Pharmaceutics, Biological Barriers to Drug absorption. Ellis Horwood Ltd. 1989; Chichester, UK.
20. Rubinstein A. Microbially controlled drug delivery to the colon Biopharm. Drug Dispos 1990; 11: 465–475.
21. Theeuwes F, Guittard GV, Wong P.S.L. 1990 US Patent, 4,904,474.
22. Muraoka M, Hu ZP, Shimokawa T, Sekino S, Kurogoshi R, KuboiY, Yoshikawa Y , Takada K . Evaluation of intestinal pressure-controlled colon delivery capsule containing caffine as a model drug in human volunteers. J Control Rel 1998; 52: 119–129.
23. SchelineRR. Metabolism of foreign compounds by gastrointestinal micro-organisms Pharmacol Rev.1973;25: 451–523.
24. Saffran M, KumarGS, Savariar C, Burnham JC, Williams F, Neekers DC.A new approach to the oral administration of insulin and other peptide drugsScience, 1986;233: 1081–1084.
25. Saffran M, Field JB, Pena J, Jones RH, Okuda Y. Oral insulin in diabetic dogs J Endocrinol.1991;131: 267–278.
26. Huang S, Fu X. Naturally derived materials-based cell and drug delivery systems in skin regeneration . Journal of Controlled Release 2010;142: 149–159
27. Witczak ZJ.Carbohydrate therapeutics ;New developments and strategies Carbohydrate drug designACS Symposium Series 2006; 93: 25–46.
28. Felt O, Buri P, Gurny R.Chitosan: a unique polysacchride for drug delivery Drug Dev. Ind. Pharm 1998; 24: 979–993.
29. Hirano S, Seino H, Akiyama Y, Nonaka I, Gebelein CG, Dunn RL (Eds.), Progress in Biomedical Polymers Plenum Press New York .1990 :283
30. Tozaki H, Komoike J, Tada C, Maruyama T, Terabe A, Suzuki T, Yamamoto A, Muranishi S.Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon J Pharm. Sci. 1997; 86: 1016–1021.
31. Tozaki H, Fujita T, Odoriba T, Terabe A,Suzuki T, Tanaka C, Okabe S,Shoo S, Muranishi S, Yamamoto A.Colon specific delivery of R68070, a new thromboxane synthase inhibitor, using chitosan capsules: therapeutic effects against 2,4,6-trinitrobenzene sulfonic acid-induced ulcerative colitis in ratsLife Science, 1999;64: 1155–1162.
32. Suzuki T, Matsumoto T, Hagino Y, Tozaki H, Yamamoto A, Muranishi S. Biodegradation and medical applications of Chitosan Hard capsules. In: Prasad, P. (Ed.). Sci. Technol. Polym. Adv. Mater. (Proc. Int. Conf. Front. Polym.Adv. Mater.). Fourth ed., New York: Plenum Press 1998;567–571.
33. Lorenzo-LamosaML, Remunan-Lopez C, Vila-Jato JL,. AlonsonM.J .Design of microencapsulated chitosan microspheres for colonic drug delivery. J Control. Release,1998; 52: 109–118.
34. Paños I, Acosta N, Heras A. New drug delivery systems based on chitosanCurrent Drug Discovery Technologies.2008; 5: 333–341.
35. Wang Q, Jonathan S, Robert D, Linhardt J.Synthesis and application of carbohydrate-containing polymersChemistry of Materials.2002;14: 3232–3244.
36. Smoum R, Rubinstein A, Srebnik M.Chitosan–pentaglycine–phenylboronic acid conjugate: A potential colon-specific platform for calcitonin. Bioconjugate Chemistry 2006;17: 1000–1007.
37. Salyers AA, Vercellotti JR, West SEH, Wilkins TD . Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon Appl. Environ. Microbiol.1977; 33: 319–32.
38. Ashford M, Fell JJ, Attwood D, Sharma HL, Woodhead PJ. An evaluation of pectin as a carrier for drug targeting to the colon. J Control Release 1993;23: 213–220.
39. Rubinstein A, Radai R, Ezra M, Pathak S, Rokem JS. In vitro evaluation of calcium pectinate: a potential colon-specific drug delivery carrier. Pharm. Res 1993; 10: 258–263.
40. Ashford M, Fell JT, Attwood D, Sharma HL, Wood PJ. Studies on pectin formulation for colonic drug delivery. J Control Release. 1994; 30: 225–232.
41. Rubinstein A, Radai A. In vitro and in vivo analysis of colon specificity of calcium pectinate formulations . Eur J Pharm Biopharm 1995; 291–295.
42. Adkin AD, KenyonCJ, LernerEI, Landau I, Strauss E, CaronD, Penhasi A, Rubinstein A, Wilding IR. The use of scintigraphy to

provide 'proof of concept for novel polysaccharide preparation designed for colonic drug delivery Pharm. Res.1997; 14: 103–107.

43. Wakerly Z, FeltJ, Attwood D, Parkins D. Studies on Amidated pectins as potential carriers in colonic. drug delivery . J Pharm. Pharmacol.1997; 49: 622–625.
44. Semde R, Amighi K, PierreD, Devleeschouwer MJ, Moes AJ.Leaching of pectin from mixed pectin/insoluble polymer films intended for colonic drug deliveryInt. J Pharm., 1998;174: 33–241.
45. Semde R, Amighi K, Devleeschouwer MJ, Moes AJ.Effect of pectinolytic enzymes on the theophylline release from pellets coated with water insoluble polymers containing pectin HM or calcium pectinateInt. J Pharm.2000; 197: 169–179.
46. Guo JH Investigation into the formation of plasticizer channels in plasticized polymer films Drug Dev. Ind. Pharm. 1994;18: 1865–1882.
47. Meshali MM, Gabr KE. Effect of interpolymer complex formation of chitosan with pectin or acacia on the release behaviour of chlorpromazine HClInt. J Pharm 1993;89: 177–181.
48. Fernandez-Hervas MJ, Fell JT . Pectin/chitosan mixtures as coatings for colon-specific drug delivery: an in vitro evaluationInt. J Pharm 1998;169 :115–119.
49. MacLeod GS, Fell JT, Collett JH, Sharma HL, Smith AM .Selective drug delivery to the colon using pectin: chitosan:hydroxypropyl methycellulose film coated tabletsInt. J Pharm. 1999;187: 251–257.
50. MacLeod GS, Fell JT, Collett JH .An in vitro investigations into the potential for biomodal drug release from petin/chitosan HPMC-coated tabletsInt. J.Pharm 1999;188:pp. 11–18.
51. Muhidinov Z, Bobokalonov J, Liu LS, Fassihi R. New delivery systems for controlled drug release from naturally occurring materialsACS Symposium Series. 2008.
52. Johnson IT, GeeJM .Effect of gel-forming gum on the intestinal unstirred layer and sugar-transport in vitroGut, 1981;22: 398–403.
53. Jain NK, Kulkarni K, Talwar N. Controlled-release tablet formulation of isoniazid.Pharmazie.1992;47: 277–278.
54. Bayliss CE,Houston AP. Degradation of guar gum by faecal bacteria Appl. Environ. Microbiol1986; 48: 626–632.
55. Tomolin J, Taylor JS, Read NW. The effect of mixed faecal bacteria on a selection of viscous polysaccharide .Nutr. Rep. Int 1989; 39: 121–135.
56. Macfarlane GT, Hay S, Macfarlane S, Gibson GR .Effect of different carbohydrates on growth, polysaccharidase and glycosidase production of *Bacteroides ovatus* in batch and continuous culture. J Appl Bacteriol 1990; 68 : 179–187.
57. Wong D, Larrabeo S, Clifford K, Tremblay J, Friend DR. USP dissolution Apparatus III (recipiocating cylinder) for screening of guar-based colonic delivery formulation . J Control Release 1997; 47: 173–179.
58. Rama Prasad YV, Krishnaiah YS, Satyanarayana S. In vitro evaluation of guar gum as a carrier for colon-specific drug delivery. J Control Release 1998;51: 281–287.
59. Krishnaiah YRS, Satyanarayana S, Rama Prasad YV, Narasimha Rao S. Gamma Scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers .J Control Release 1998;55: 245–252.
60. Krishnaiah YRS, Satyanarayana S, Rama Prasad YV. Studies of guar gum compression-coated 5-aminosalicylic acid tablets for colon-specific drug delivery. Drug Dev Ind Pharm 1999; 25: 651–657.
61. Pinhassi RI, Assaraf YG, Farber S, Stark M, Ickowicz D, Drori S. Arabinogalactan-folic acid-drug conjugate for targeted delivery and target-activated release of anticancer drugs to folate receptor-overexpressing cells . Biomacromolecules 2010; 11: 294–303.
62. Hultin E, Nordstom L . Investigation on dextranase-1. On the occurance and the assay of dextranase .Acta Chem Scand 1949 ;11: 1405–1417.
63. Ingelman E. Enzymatic breakdown of dextran. Acta Chem Scand 1948; 2 : 803–812.
64. Rosenfeld EL, Lukomskaya IS. The splitting of dextran and isomaltose by animal tissue. Clin Chem Acta 1957; 2 : 105–113.
65. Hehre EJ,Sery TW. Dextran splitting anaerobic bacteria from the human intestine . J Bacteriol 1952; 63: 424–426.
66. Aberg B. Breakdown of dextran by human faces. Scand J Clin Lab Invest 1953; 5 :37–38

67. Drasar BS, Hill MJ. Human Intestinal Flora. Academic Press, New York 1974 ; 15–43.

68. Larsen C, Harboe E, Johansen M, Olsen HP. Macromolecular prodrugs XVI. Colon targeted delivery – a comparison of rate of release of naproxen from dextran ester prodrugs in homogenates of various segments of the pig gastrointestinal tract . Pharm Res 1989; 6 : 995–999.

69. Larsen C, Jensen BH, Olesen HP. Bioavailability of ketoprofen from orally administered ketoprofen–dextran ester prodrug in the pig .Acta Pharm Nord 1991; 3: 71–76.

70. McLeod AD, Friend DR, Tozer TN. Synthesis and chemical stability of glucocorticoid–dextran esters: potential prodrugs for colon-specific delivery . Int J Pharm 1993; 92 :105–114.

71. McLeod AD, Friend DR, Tozer TN. Glucocorticoid–dextran conjugates as potential prodrugs for colon-specific delivery: hydrolysis in rat gastrointestinal tract contents. J Pharm Sci 1994; 83: 1284–1288.

72. McLeod AD, Tolentino L,Tozer TN. Glucocorticoid–dextran conjugates as potential prodrugs for colon-specific delivery: steady-state pharmacokinetics in the rat. Biopharm. Drug Dispos 1994; 15: 151–161.

73. McLeod AD, Fedorak RN, Friend DR, Tozer TN, Cui N.A glucocorticoid prodrug facilities normal mucosal function in rat colitis without adrenal suppression.Gastroenterology 1994;106: 405–410.

74. JungYJ, Lee JS, Kim HH, Kim YK, Kim YM.Synthesis and properties of dextran-5-amino salicyclic acid ester as a potential colon-specific prodrug of 5-amino salicylic acid. Arch Pharm Res 1998;21: 179–186.

75. Brondsted H, Hovgaard L, Simonsen L. Dextran hydrogels for colon specific drug delivery III. In vitro and in vivo degradation. STP Pharm1995;5: 60–64.

76. Brondsted H, Hovgaard L , Simonsen L. Dextran hydrogels for colon specific drug delivery IV. Comparative release mechanisms of hydrocortisone and prednisolone phosphate. STP Pharm 1995;5: 65–69.

77. Chiu HC, Hsiue GH, Lee YP, Huang LW. Synthesis and characterization of pH-sensitive dextran hydrogels as a potential colon-specific drug delivery system. J Biomater. Sci Polym Ed1999; 10: 591–608.

78. Larrosa M, Tomé-Carneiro J, Yanez-Gascon MJ, Alcántara D, Selma MV, Beltrán D .Preventive oral treatment with resveratrol pro-prodrugs drastically reduce colon inflammation in rodents . Journal of Medicinal Chemistry 2010;53: 7365–7376.

79. Hirsch S, Binder V, Kolter K, Kesselhut JF, Bauer KH. Lauroyldextran as a coating material for site-specific drug delivery to the colon: in-vitro dissolution of coated tablets. Proc Int Symp Control Rel Bioact Mater 1997; 24:379–380.

80. Hirsch S, Binder V, Schehlmann V, Kolter K, Bauer KH. Lauroyldextran and cross-linked galactomannan as coating materials for site-specific drug delivery to the colon. Eur J Pharm Biopharm 1999; 47: 61–71.

81. Van Loo J, Coussement P, De Leenheer L, Hoebregs H, Smits G.On the presence of inulin and oligofructose as natural ingredients in the western diet. Cri Rev Food Sci Nutr 1995;36 : 525–552.

82. Roberfroid MB. Dietary fiber, inulin and oligofructose: a review comparing their physiological effects. Crit Rev Food Sci Nutr1993; 33: 103–148.

83. McKellar RC, Modler HW. Metabolism of fructooligosaccharides by *Bifidobacterium* spp. Appl Microbiol Biotechnol 1989; 31: 537–541.

84. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125: 1401–1414.

85. Vervoort L, Kinget R. In vitro degradation by colonc bacteria of inulin HP incorporated in Eudragit filmsInt. J Pharm 1996;129: 185–190.

86. VervoortL , Van den Mooter G, Augustijins P, Busson R, Toippet S, Kinget R. Inulin hydrogels as carriers for colonic drug targeting: I. Synthesis and characterization of methacrylated inulin and hydrogel formation. Pharm Res 1997; 14: 1730–1737.

87. Vervoort L, Van den Mooter G, Augustijns P, Kinget R. Inulin hydrogels I Dynamic and equilibrium swelling properties. Int J Pharm1998; 172: 127–135.

88. Vervoort L, Rambant P, Van den Mooter G, Augustijns P, Kinget R.Inulin hydrogels II. In vitro degradation study. Int J Pharm 1998;172: 137–145.

89. VervoortL ,Vinckier I, Moldenaers P, Van den Mooter G, Ahgustigns P, Kinget R . Inulin hydrogels as carriers for colonic drug targeting Rheological charaterization of the hydrogel formation and the hydrogel network .J Pharm Sci 1999;88: 209.

90. Wastenson A.Properties of fractionated chondritin sulphate from ox nasalSepta. Biochem J 1971;122: 477–485.

91. Salyers AA, Brien M . Cellular location of enzymes involved in chondroitin sulfate breakdown by *Bacteroides thetaiotaomicron* .\J Bacteriol 1980;143: 772–780.

92. Sintov A, Di-capua N, Rubinstein A. Cross-linked chondroitin sulphate: characterization for drug delivery purposes Biomaterials. 1995;16: 473–478.

93. Leloup VM, Colonna P, Ring SG. α-Amylase adsorption on starch crystallites. Biotechnol Bioeng 1991;38: 127–134.

94. Englyst HN, MacFarlane GT. Breakdown of resistant and readily digestible starch of human gut bacteria . J Sci Food Agric 1986;37 : 699–706.

95. Ring SG, Gee JM, Whittam M, Orford P, Johnson IT. Resistant starch Its chemical form in food stuffs and effects on digestibility in vitro. Food Chem 1988; 28 : 97–109.

96. Milojevic S , Newton JM, Cummings JH, Gibson GR, Botham RL, Ring SG, Stockham M, Allwood MC . Amylose the new perspectivein oral drug delivery to the human large intestineS. TP Pharm Sci 1995; 51: 47–53.

97. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, Ring SG, Stockham M, Allwood MC. Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using 5-aminosalicylic acid pellets. J Control Release 1996;38:75–84.

98. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, Ring SG, Stockham M, Allwood MC. Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using glucose pelletsJ. Control Release 1996;38: 85–94.

99. Cummings JH, Milojevic S, Harding M, Coward WA, Gibson GR, Botham RL, Ring SG, Wraight EP, Stockham MA, Alwood MC, Newton JM. In vivo studies of amylose- and ethycellulose-coated [^{13}C]glucose microspheres as a model for drug delivery to the colon. J Control Release 1996;40: 123–131.

100. SiewLF, Basit AW, Newton JM. The properties of amylose–ethylcellulose films cast from organic based solvents as potential coating for colonic drug delivery. Eur J Pharm Sci 2000;11: 133–139.

101. Siew LF, Basit AW, Newton JM. The potential of organic based amylose ethylcelulose film coatings as oral colon-specific drug delivery systems. AAPS Pharm Sci Tech 2000b;22.

102. Andersen GH, Robbins FM, Domingues FJ, Moores RG, Long CL. The utilization of schardinger dextrins by the rat. Toxicol Appl Pharmacol 1983; 5: 257–266.

103. Flourie B, Molis C,Archour L, Dupas H, Hatat C, Rambaud JC. Fate of β-cyclodextrin in the human intestine. J Nutr 1993;123: 676–680.

104. Uekama K, Minami K, Hirayama F. 6^{A}-O-[(4-Biphenylyl)acetyl)]-α-,-β-, and -γ-cyclodextrins and 6^{A}-deoxy-6^{A}[(4-biphenylacetyl)]amino]-α-,-β-, and -γ-cyclodextrins: potential prodrugs for colon-specific delivery. J Med Chem1997;40: 2755–2761.

105. Shah N, Shah T, Amin A. Polysaccharides: A targeting strategy for colonic drug delivery Expert Opinion on Drug Delivery.2011;8: 779–796.

106. Shun YL, Ayres JW. Calcium Alginate beads as core carriers of 5-Aminosalicylic acid. Pharm Res 1992; 9: 714–790.

107. Kiyoung L, Kun N, Yueim K. Polysaccharides as a drug coating polymer Polym. Prep 1990;40: 359–360.

108. Liang XF, Wang HJ, Luo H, Tian H, Zhang BB, Hao LJ. Characterization of novel multifunctional cationic polymeric liposomes formed from octadecyl quaternized carboxymethyl chitosan/cholesterol and drug encapsulation. Langmuir 2008;24: 7147–7153.

109. Liu L, Fishman ML, Kost J, Hicks KB. Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials 2003; 24: 3333–3343.

110. Vaidya A, Agarwal A, Jain A, Agrawal RK, Jain SK.Bioconjugation of polymers: A novel platform for targeted drug deliveryCurrent Pharmaceutical Design. 2011;17:1108–1125.

111. RinaudoM. Chitin and chitosan: Properties and applicationsProgress in Polymer Science. 2006;31: 603–632.

112. Roos AA, Edlund U, Sjöberg J, Albertsson AC, Stålbrand H.Protein release from galactoglucomannan hydrogels: Influence of substitutions and enzymatic hydrolysis by beta-mannanase. Biomacromolecules 2008; 9: 2104–2110.

113. Takei T, Sato M, Ijima H, Kawakami K.In situ gellable oxidized citrus pectin for localized delivery of anticancer drugs and prevention of homotypic cancer cell aggregationBiomacromolecules 2010; 11: 3525–3530.

114. Galbiati A, Tabolacci C, Morozzo Della Rocca B, Mattioli P, Beninati S, Paradossi G.Targeting tumor cells through chitosan-folate modified microcapsules loaded with camptothecin . Bioconjugate Chemistry2011;22:1066–1072.

115. Casadei MA, Pitarresi G, Calabrese R, Paolicelli P, Giammona G. Biodegradable and pH-sensitive hydrogels for potential colon-specific drug delivery characterization and in vitro release studies. Biomacromolecules 2008;9: 43–49.

116. KopeekJ .Biomaterials and drug delivery: Past, present, and futureMolecular Pharmaceutics 2010;7 : 922–925.

117. Nichifor M, Mocanu G. Polysaccharide-drug conjugates as controlled drug delivery systems polysaccharides for drug delivery and pharmaceutical applications ACS Symposium Series 2006;934: 289–303.

118. Mandal AS, Biswas N, Karim KM, Guha A, Chatterjee S, Behera M.Drug delivery system based on chronobiology—A review. Journal of Controlled Release 2010;147: 314–325.

119. Al-Tabakha MM. HPMC capsules: Current status and future prospects .Journal of Pharmacy and Pharmaceutical Sciences 2010;13: 428–442.

120. Caraballo I. Factors affecting drug release from hydroxypropyl methylcellulose matrix systems in the light of classical and percolation theories .Expert Opinion on Drug Delivery 2010;7 : 1291–1301.

121. Li CL, Martini LG, Ford JL, Roberts M.The use of hypromellose in oral drug delivery. Journal of Pharmacy and Pharmacology 2005; 57:533–546.

122. Raymond R, Paul JS, Siân CO.Handbook of pharmaceutical excipients Part 3. American Pharmacists Association, Pharmaceutical Press 2006; 354.

123. Sarfaraz N.Handbook of pharmaceutical manufacturing formulations. CRC Press, Boca Raton FL 2004; 275–276.

124. Bassi P, Kaurp G . Modulation: A mechanism to obtain pH-independent drug release. Expert Opinion on Drug Delivery 2010; 7: 845–857.

125. Kosaraju SL. Colon targeted delivery systems: Review of polysaccharides for encapsulation and delivery. Critical Reviews in Food Science and Nutrition 2005;45 : 251–258.

126. Siepmann F, Siepmann J, Walther M, MacRae RJ, Bodmeier R. Polymer blends for controlled release coatings. Journal of Controlled Release 2008;125 : 1–15.

127. Ring SG, Archer DB, Allwood MC, Newton JM. *Delayed release formulations*. 1991; WO 91/07946.

128. Milojevic S, Newton JM , Cummings JH , Gibson G, Botham RL , Ring SG, Allwood MC, Stockham M. In vitro and in vivo evaluation of amylose coated pellets for colon-specific drug delivery. Proceedings of the International Symposium on Controlled Release of Bioactive Materials 1993;20: 288–289.

129. Allwood M, Archer D, Ring S. *Delayed release formulations*. 1989; WO 89/11269

130. Newton JM, SiewLF. *Controlled release formulations*. 1999;WO 99/21536.

131. Newton JM, Leong C. *Low temperature coatings*. 1990;WO 99/25325.

132. Ugurlu T, Turkoglu M, Gurer US, Akarsu BG . Colonic delivery of compression coated nisin tablets using pectin/HPMC polymer mixture. European Journal of Pharmaceutics and Biopharmaceutics 2007;67: 202–210.

133. Hodges LA, Connolly SM, Band J, Mahony OB, Ugurlu T, Turkoglu M .Scintigraphic evaluation of colon targeting pectin-

HPMC tablets in healthy volunteers. International Journal of Pharmaceutics 2009;370: 144–150.

134. Kim JH, Fassihi R. Application of a binary polymer system in drug release rate modulation 1. Characterization of release mechanism. Journal of Pharmaceutical Sciences 1997; 86(2) : 316–322.

135. Kim JH, Fassihi R. Application of a binary polymer system in drug release rate modulation.2. Influence of formulation variables and hydrodynamic conditions on release kinetics. Journal of Pharmaceutical Sciences 1997; 86 (3): 323–328.

136. Kim JH, Fassihi R. A new ternary polymeric matrix system for controlled drug delivery of highly soluble drugs: I. Diltiazem hydrochloride. Pharmaceutical Research 1997;14: 1415–1421.

137. l drug release from pectin/chitosan/HPMC-coated tablets. International Journal of Pharmaceutics 1999;188 (1): 11–18.

138. Macleod GS, Fell JT, Collett JH, Sharma HL, Smith AM. Selective drug delivery to the colon using pectin: chitosan: hydroxypropylmethylcellulose film coated tablets. International Journal of Pharmaceutics 1999;187 (2): 251–257.

139. Chaudhary A, Tiwari N, Jain V, Singh R. Microporous bilayer osmotic tablet for colon- specific delivery. European Journal of Pharmaceutics and Biopharmaceutics 2011;78: 134–140.

140. Nunthanid J, Huanbutta K, Luangtana-Anan M, Sriamornsak P, Limmatvapirat S, Puttipipatkhachorn S. Development of time, pH and enzyme-controlled colonic drug delivery using spray-dried chitosan acetate and hydroxypropyl methylcellulose. European Journal of Pharmaceutics and Biopharmaceutics 2008; 68: 253–259.

141. Priscileila CF, Giselle FO, Flávia Cristina SC, Raul CE. In vitro characterization of coevaporates containing chitosan for colonic drug delivery. Carbohydrate Polymers 2009;78: 557–563.

142. N. Mennini N, S. Furlanetto S, M. Cirri M, P. Mura P. Quality by design approach for developing chitosan-Ca-alginate microspheres for colon delivery of celecoxib-hydroxypropyl-β-cyclodextrin-PVP complex. European Journal of Pharmaceutics and Biopharmaceutics 2012;80 : 67–75.

143. Freire F, Podczeck, Ferreira D, Veiga F, Sousa J, Pena A. Assessment of the in vivo drug release from pellets film-coated with a dispersion of high amylose starch and ethylcellulose for potential colon delivery. Journal of Pharmacy and Pharmacology 2010; 62 : 55–61.

144. Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Dubreuil L. Colon targeting with bacteria-sensitive films adapted to the disease state. European Journal of Pharmaceutics and Biopharmaceutics 2009; 73: 74–81.

145. Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Dubreuil L. Novel polymeric film coatings for colon targeting: Drug release from coated pelletsEuropean. Journal of Pharmaceutical Sciences 2009; 37: 427–433.

146. Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Dubreuil L.Enzymatically degraded Eurylon 6 HP-PG: Ethylcellulose film coatings for colon targeting in inflammatory bowel disease patients . Journal of Pharmacy and Pharmacology 2010; 62 : 1676–1684.

147. Ali Asghar LF, Azeemuddin M, Jain V, Chandran S. Design and in vitro evaluation of formulations with pH and transit time controlled sigmoidal release profile for colon-specific delivery . Drug Delivery 2009;16: 295–303.

148. Bigucci F, Luppi B, Cerchiara T, Sorrenti M, Bettinetti G, Rodriguez L .Chitosan/pectin polyelectrolyte complexes: Selection of suitable preparative conditions for colon-specific delivery of vancomycin . European Journal of Pharmaceutical Sciences 2008; 35: 435–441.

149. Bigucci F, Luppi B, Monaco L, Cerchiara T, Zecchi V. Pectin-based microspheres for colon-specific delivery of vancomycin. Journal of Pharmacy and Pharmacology 2009; 61: 41–46.

150. Giselle FO, Priscileila CF, Livia QC, Raul CE. Chitosan–pectin multiparticulate systems associated with enteric polymers for colonic drug delivery Carbohydrate Polymers 2010;82 : 1004–1009.

151. Haupt S, Zioni T, Gati I, Kleinstern J, Rubinstein A. Luminal delivery and dosing considerations of local celecoxib administration to colorectal cancer. European Journal of Pharmaceutical Sciences 2006;28: 204–211.

152. Ravi SV, Pramod Kumar TM. Influence of natural polymer coating on novel colon targeting drug delivery system . Journal of Materials Science Materials in Medicine 2008;19 : 2131–2136.

153. Tugcu-Demiröz F, Acartürk F, Takka S. Investigation of colon-specific dosage forms of ondansetron prepared with natural polymers. Pharmazie 2006; 61: 916–919.

154. Glavas Dodov M, Calis S, Crcarevska MS, Geskovski N, Petrovska V, Goracinova K. Wheat germ agglutinin-conjugated chitosan-Ca-alginate microparticles for local colon delivery of 5-FU: Development and in vitro characterization. International Journal of Pharmaceutics 2009; 381: 166–175.

155. Laroui H, Dalmasso G, Nguyen HT, Yan Y, Sitaraman SV, Merlin D. Drug-loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse modelGastroenterology 2010; 138 : 843–853.

156. Lin WC, Yu DG, Yang MC. pH-sensitive polyelectrolyte complex gel microspheres composed of chitosan/sodium tripolyphosphate/dextran sulfate: Swelling kinetics and drug delivery properties Colloids and Surfaces B: Biointerfaces 2005; 44: 143–151.

157. Wakerly Z, Fell JT, Attwood DA, Parkins D. Pectin/ethylcellulose film coating formulations for colonic drug delivery. Pharmaceutical Research 1996; 13 (8): 1210–1212.

158. Arwidsson H, Hjelstuen O, Ingasen D, Graffner C. Properties of ethylcellulose films for extended release II. Influence of plasticizer content and coalescence conditions when using aqueous dispersion. Acta Pharmaceutica Suecica 1991; 3: 65–70.

159. Lippold BC, Lippold BH, Sutter BK, Gunder W. Properties of aqueous, plasticizer-containing ethylcellulose dispersion and prepared films in respect to the production of oral extended release formulations. Drug Development and Industrial Pharmacy 1990;16: 1725–1747.

160. Parikh NH, Portery SC, Rohera BD. Tensile properties of free films cast from aqueous ethycellulose dispersions Pharmaceutical Research 1993; 10: 810–815.

161. Bodmeier R, Paeratakul O. Mechanical properties of dry and wet cellulosic and acrylic films prepared from aqueous colloidal polymer dispersions used in the coating of solid dosage Forms. Pharmaceutical Research 1994; 11: 882–888.

162. Obara S, Mc Ginity JW. Properties of free films prepared from aqueous polymers by spraying technique. Pharmaceutical Research1994; 11: 1562–1567.

163. Obara O, Mc Ginity JW. Influence of process variables on the properties of free films prepared from aqueous polymeric dispersions by a spray technique. International Journal of Pharmaceutics 1995; 126: 1–10.

164. Semdé R. *Etude des pectines en vue de leur utilisation pour la délivrance spécifique des médicaments dans le colon après administration orale*, PhD Thesis Université libre de Bruxelles 1999.

165. Ji C, Xu H, Wu W. In vitro evaluation and pharmacokinetics in dogs of guar gum and Eudragit FS30D-coated colon-targeted pellets of indomethacin. Journal of Drug Targeting 2007; 15: 123–131.

166. Soni ML, Namdeo KP, Jain SK, Gupta M, Dangi JS, Kumar M. pH-enzyme di-dependent chronotherapeutic drug delivery system of theophylline for nocturnal asthma . Chemical and Pharmaceutical Bulletin 2011; 59: 191–195.

167. Ji CM, Xu HN, Wu W. Guar gum as potential film coating material for colon-specific delivery of fluorouracil . Journal of Biomaterials Applications 2009; 23: 311–329.

168. Jain A, S Jain SK, Ganesh N, Barve J, A Beg AM. Design and development of ligand-appended polysaccharidic nanoparticles for the delivery of oxaliplatin in colorectal cancer. Nanomedicine 2010; 6: 179–190.

169. Adkin D, Davis S, Sparow R, Wilding J. Colonic transit of different sized tablets in healthy subjects. Journal of Controlled Release1993; 23:147–153.

170. Kotagale N, Maniyar M, Somvanshi S, Umekar M, Patel CJ. Eudragit-S, Eudragit-L and cellulose acetate phthalate coated polysaccharide tablets for colonic targeted delivery of azathioprine. Pharmaceutical Development and Technology 2010; 15: 431–437.

171. Jain V, Jain D, Singh R. Factors affecting the morphology of Eudragit S-100 based microsponges bearing dicyclomine for colonic delivery. Journal of Pharmaceutical Sciences 2010; 100:1545–1552.

172. Ibekwe VC, Khela MK, Evans DF, Basit AW.A new concept in colonic drug targeting: A combined pH-responsive and bacterially-

triggered drug delivery. TechnologyAlimentary Pharmacology and Therapeutics 2008; 28: 911–916.

173. Dubey R, Omrey P, Vyas SP, Jain SK. Development and characterization of colon specific drug delivery system bearing 5-ASA and Camylofine dihydrochloride for the treatment of ulcerative colitis. Journal of Drug Targeting 2010; 18: 589–601.

174. M.L Lorenzo-Lamosa ML, C Remuñán-Lopez C. Design of microencapsulation chitosan microspheres for colonic drug delivery. Journal of Controlled Release 1998; 52: (1–2) 109–118.

175. J.L Rasic JL, J.A Kurmann JA. Bifidobacteria and their role — Microbiological, nutritional, physiological, medical, and technological aspects and bibliographyExperientia Supplementum 1983; 39: 1–295.

176. Medicines.org.uk [homepage on the Internet]. Leatherhead, United Kingdom:Clipper®.Availablefrom:http://www.medicines.org.uk/emc/medicine/21329#PRODUCTINFO.

177. Sang PJ, Sim SU, Yeon J. Inventors, GL PHARMTECH CORP (Seongnam-si, Gyeonggi-do, KR) assignee. Oral Sustained-Release Triple Layer Tablet, United States Patent Application 20100040681, 02/18/2010.

178. Andrea G, Matteo C, Alberto C, Anastasia Anna F, Maria S. Edvige & Lucia Z. Inventors UNIVERSITA' DEGLI STUDI DI MILANO Milano, IT, assignee, Pharmaceutical Dosage Forms For Time-Specific Drug Delivery, European Patent, EP2009/005370, 01/28/2010.

179. Gazzaniga B, Palugan L, Foppoli A, Sangalli ME. Oral pulsatile delivery systems based on swellable hydrophilic polymers. European Journal of Pharmaceutics and Biopharmaceutics2008; 68: 11–18.

180. Gazzaniga M, Cerea A, Cozzi A, Foppoli L, Zema A, Maroni ME. Injection-molded swellable/erodible capsular devices intended for oral pulsatile delivery Proceedings of 36th annual meeting & exposition of the controlled release society, Copenhagen, Denmark, 18–22 July 2009.

181. Staniforth JN, ABaichwal AR. TIMERx: Novel polysaccharide composites for controlled/programmed release of drugs in the gastrointestinal tract Expert Opinion on Drug Delivery 2005; 2: 587–595.

182. Watts P, Smith A. TARGIT technology: Coated starch capsules for site-specific drug delivery into the lower gastrointestinal tract. Expert Opinion on Drug Delivery 2005; 2: 159–167.