

Colon Targeting Of Drugs: The Factors, Targeting Approaches And Evaluation Strategies.

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Abstract: Targeting drugs to colon has been one of the major areas of scientific interest for researchers and has been thoroughly investigated since the last few decades. Colon targeting of drugs has helped to reduce the toxic effects of drugs due to systemic absorption, to increase bioavailability of drugs which are affected in the upper parts of the gastrointestinal tract, to reduce dose of drugs and to offer better therapeutic management of the colonic diseases like ulcerative colitis, chron's disease or colon cancer by providing local action at the site of the affected area. Several approaches have been investigated by research scientists to target drug release to the colon. However challenges pertaining to specific drug release in the colon, avoiding premature drug release or development of specific evaluation methods for the delivery systems have been major areas of concern. In this review article we would go through the various delivery approaches to target colon and then discuss the several factors that may affect the behavior of the dosage forms. We would also take a look at the various in-vitro and in-vivo methods for evaluation of the delivery systems.

Key words: Colon targeting, Factors, Bioavailability, Targeting approaches, Evaluation.

Introduction

Drugs have been targeted to the colon for the treatment of several diseases like ulcerative colitis, amoebiasis, chron's disease and colorectal cancer. Such conditions require local action of the drugs rather than systemic absorption. Several drug delivery systems have been designed to release drugs specifically in the large intestine to improve therapeutic efficacy, to reduce dose of drugs, and amount to low systemic absorption resulting in fewer side effects. Several proteins or peptides which are degraded in the stomach and small intestine due to the abundance of digestive enzymes are also targeted to the colon for systemic absorption and improved therapeutic efficacy^[1]. However, several challenges are still being faced by researchers for selectively targeting drugs to the colon and newer approaches for drug targeting are being developed.

Targeting Approaches for Colon Drug Delivery

pH dependent systems:

Drug delivery from pH dependent systems depends on the varying pH conditions of the gastrointestinal tract which ranges from highly acidic to alkaline pH. Table 1 illustrates the various pH values of different parts of the gastrointestinal tract^[1,2].

Table 1: pH values of different parts of the gastrointestinal tract

Region of the gastrointestinal tract	pH
Stomach	1.2
Duodenum	6.6
Ileum	7.5
Caecum	6.4
Transverse colon	6.6
Descending colon	7.0

pH dependent systems are formulated mainly with the help of pH sensitive coating polymers which are insoluble in acidic pH but soluble in alkaline pH. Important examples include cellulose acetate phthalate, shellac and eudragits. Sinha et al evaluated various coating polymers including eudragit S100, cellulose acetate phthalate, shellac and ethyl cellulose, for colon specific drug delivery. Comparative dissolution data revealed that, of all the polymers used, a 3% m/m coat of shellac was most suitable for colonic drug delivery^[7]. Akhgari et al studied the effects of the ratio of Eudragit S100: Eudragit L100 and the coating level on indomethacin release from pellets to optimize coating formulations for colonic drug delivery. They reported that the coating formulation consisting of Eudragit S100: Eudragit L100 in 4:1 ratio at 20% coating level has potential for colonic drug delivery from indomethacin loaded pellets^[8].

Sustained release systems:

It is understood that the pH dependent drug delivery systems for colon targeting should not release the drug in stomach and small intestine, but the drug release should start in the caecum and thereafter the drug should be released in a manner that is required of the delivery system to ensure maximum therapeutic efficacy. For example, if maximum part of the colon is affected, the delivery system should ensure sustained release of the drug in the colon rather than immediate release. Sustained release of the drug from the delivery device is often favorable after the device has reached the colon. If immediate release of drug takes place all at a time, the high concentrations of the locally released drug will act on both inflamed as well as healthy tissues and may, therefore, cause local irritations. Sustained release dosage forms for colon targeting may be classified as single-unit or multiple unit dosage forms. Single unit dosage forms are formulated as matrix tablets with extended release polymers, coated with pH dependent polymers. When the coating dissolves in the upper intestine, the core matrix tablet releases the drug in a sustained manner in the colon. The multiple unit dosage forms consist of a number of single unit dosage forms, in the form of pellets, granules or microspheres, enclosed within a capsule or tablet. When the tablet or capsule disperses to release its contents, each of the particulates behaves as single unit dosage form.

Multiparticulate drug delivery systems:

Modified release formulations containing multiparticulates like pellets, granules or microspheres enclosed in capsules or compressed into tablets have been designed in order to give sustained release over the entire length of the colon, avoiding dose dumping at a certain region in the colon. They are less likely to be affected by food, ensure consistent absorption and upon disintegration of capsule shell or tablet, they ensure uniform distribution over the affected parts of the colon to maximize therapeutic efficacy^[2]. The tablets or capsules are coated with an enteric polymer which protects the system in the stomach and small intestine. Thereafter, the enclosed or compressed particulates are released in the distal ileum or caecum and spread uniformly throughout the region. Each particle or pellet serves as an individual delivery device and releases the drug in a controlled manner.

Rhodes and Evans developed a delayed release formulation in which enteric coated granules containing drug were enclosed in an enteric coated capsule shell. The enteric coating was done with a polymer which would dissolve above pH 7. When the capsule shell opens up in the small intestine, the coating of the granules prevents drug release until the granules reach the ileum or caecum, and thereafter, a sustained drug release takes place in the colon^[9].

Siepmann et al prepared 5-Aminosalicylic acid loaded beads and coated them with different blends of nutriose:ethylcellulose. In vitro release studies were performed in media containing fresh fecal slurries of patients of inflammatory bowel diseases and in culture media containing colonic bacteria. The drug release from the coated pellets was found to increase significantly in media containing fecal slurries and thereafter the release continued in a sustained manner. Nutriose used in this study is a starch derivative which is claimed to be selectively degraded by enzymes produced by colonic bacteria of ulcerative colitis and chron's disease affected patients^[10].

Microbial triggered systems:

The colonic fluid contains 400 different species of bacteria and carbohydrates like non-starch polysaccharides are one of the primary foods of such bacteria. The stomach or small intestine does not have such vast number and variety of bacterial species as found in the colon. Therefore drug delivery systems coated or formulated with such polysaccharides which serve as food for the bacterial population present in colonic fluid would help to achieve site specific drug delivery. Such systems may not be affected by the varying pH conditions of different individuals. Based on this concept, several polysaccharides have been investigated for site specific delivery in the colon. Table 2 lists out some of the polysaccharides together with the bacterial species which break them down^[11].

Table 2: Polysaccharides and bacterial species responsible for their breakdown

Polysaccharide	Bacterial species
Amylose	Bacteroids , Bifidobacterium
Arabinogalactone	Bifidobacterium
Chitosan	Bacteroids
Chondroitin sulfate	Bacteroids
Cyclodextran	Bacteroids
Dextran	Bacteroids
Guar gum	Bacteroids, Ruminococcus
Pectin	Bacteroids, Bifidobacterium, Eubacterium
Xylan	Bacteroids, Bifidobacterium

Formulations containing various polysaccharides have been investigated for site specific delivery in the colon. Nasra et al developed matrix, multilayer and compression coated tablets of metronidazole using pectin as carrier. In vitro release studies indicated that matrix and multilayer tablets failed to control drug release in the stomach or intestinal pH conditions, but pectin containing compression coated formulations were able to protect the tablet cores from premature drug release in stomach or intestinal pH conditions^[12]. Gupta et al developed pectin matrix tablets containing 5-Fluorouracil coated with eudragit S100 and inulin and evaluated drug release in colonic environment. The best release profile ($8.5 \pm 2.58\%$ of drug release in first 5hrs.) was exhibited by the matrix tablets containing 75% pectin by weight and coated with combination of eudragit S100 and inulin in the ratio of 60%:40% to obtain a weight gain of 12.91%.^[13] Kaushik et al prepared 5-Fluorouracil loaded chitosan microspheres for colon targeting and evaluated cytotoxicity in vitro on human colon cancer cell lines. In vitro dissolution studies in simulated colonic fluid (pH 7.0) with 4% rat caecal content revealed $61.65 \pm 2.96\%$ drug release in 24 hours. Cytotoxicity study indicated that the microspheres prolonged the cytotoxic effect on HT-29 colon cancer lines in comparison to free drug^[14]. Paharia et al prepared eudragit coated pectin microspheres for colon targeting of 5-Fluorouracil and evaluated in vitro drug release in gastrointestinal fluids. The release rate was much slower in acidic medium but increased quickly at pH 7.4. Organ distribution study in albino rats established the colon targeting potential of the microspheres^[15]. Sinha et al prepared rapidly disintegrating core tablets of 5-Fluorouracil and compression coated with a mixture of xanthum gum and guar gum in varying proportions and evaluated for drug release in simulated colonic medium with 2% and 4% rat caecal contents.

The formulation prepared from xanthum gum and guar gum in the ratio of 10:20 released $67.2 \pm 5.23\%$ in presence of 2% and $80.34 \pm 3.89\%$ in presence of 4% rat caecal contents^[16]. Lorenzo-Lamosa et al developed chitosan microcores entrapped within acrylic microspheres with sodium diclofenac as model drug by solvent evaporation method. No release was observed at acidic pH. However after reaching the eudragit pH solubility, a continuous release for a variable time (8-12 hrs.) was achieved^[17]. Krishnaiah et al developed 5-Fluorouracil core tablets and compression coated with 60%, 70% and 80% of guar gum. In vitro drug release studies revealed 2.5-4% drug release in simulated gastrointestinal fluids. The formulation coated with 80% guar gum released only 2.38% drug in gastrointestinal fluids and 41% release in simulated colonic fluid with 4% rat caecal content in 24 hours^[18]. Shivani et al prepared chitosan microspheres and coated with eudragit S100 and studied drug release in changing pH media. The coated microspheres showed no drug release in simulated gastric fluid, negligible release of 8% in simulated intestinal fluid and substantial release of 95% in simulated colonic fluid^[19]. Kumar et al formulated matrix tablets with pectin as carrier and were coated with inulin followed by shellac. In vitro release studies showed that the tablets have limited drug release in stomach and small intestine and maximum release in the colonic environment^[20]. Ravi et al developed tablets using chitosan, guar gum as carriers and ditiagem hydrochloride as model drug and coated the tablets with inulin as inner coat and shellac as outer coat. The in-vitro release studies revealed that the tablets controlled drug release in the stomach and small intestine and released maximum drug in the colon. They further concluded that chitosan was a suitable carrier for colon targeting^[21].

Time dependent systems:

These type of systems use rate controlling high viscosity polymers to attain minimum release of drug in the upper parts of the gastrointestinal tract, that is, the stomach and the small intestine, and release maximum amount of the drug in the colonic region based on the long colonic residence time. The drug delivery systems require on an average 5 to 6 hours to pass the stomach and small intestine (2-3 hours in the stomach and 3 hours in the small intestine). However, the transit time for drug delivery systems may vary between different individuals and is dependent on type and amount of food taken. Peristaltic movement of the gastrointestinal tract can also cause significant changes in the gastrointestinal transit of drug delivery devices. Again, certain disease conditions like diarrhea- predominant irritable bowel syndrome may cause much increase in the rate of transit and may cause the defecation of the drug delivery device before sufficient drug release has taken place. Due to all these factors time dependent systems are not very reliable and often combinations of pH and time dependent systems are developed which places a better approach for specific drug targeting in the colon. In such combined systems, the drug delivery device is coated with a pH dependent polymer such that the coating dissolves in the small intestine and thereafter, the inner core, made up of release sustaining polymers release lesser amount of drug in the small intestine and maximum possible drug in the colon. Patel et al developed such a time and pH dependent system for colon specific delivery of mesalamine in the colon. The core tablet of mesalamine was coated with a pH-independent hydrophilic polymer, hydroxypropylmethylcellulose. The core tablet was then further coated with a pH-dependent methacrylic acid polymer, eudragit S100. From in-vitro evaluation, it was revealed that the system could exhibit site-specific drug delivery to the colon^[22]. Obitte et al prepared metronidazole granules with methylcellulose as matrix former and filled into capsules which were given coats of eudragit L100 and Landolphia owariensis latex subsequently. On evaluation, it was found that the greatest quantity of drug release took place at pH 7.4 over 9-20hrs^[23]. Calanchi et al developed a formulation by coating the drug core with two membranes, one having pH dependent solubility and the other insoluble but permeable to intestinal juices. At pH less than 5.5, no drug was released. But at higher pH, the pH dependent membrane dissolves and the release of the drug starts. At this point the second membrane consisting of pH independent polymer slows down and controls the dissolution of the drug in the small intestine and colon^[24].

Pressure controlled systems:

Few novel devices for colon targeting have been developed based on the fact that the luminal pressure from peristalsis is higher in the colon because the viscosity of the colonic contents is high while the fluid content is much less compared to the upper parts of the gastrointestinal tract including the stomach or small intestine. The pressure controlled systems are developed such that they are able to withstand the luminal pressure in the upper parts of the gastrointestinal tract and the membrane enclosing the drug opens up under the higher luminal pressure in the colon. By controlling the thickness of the membrane, the collapse time can be manipulated. (2) Shibata et al developed pressure-controlled colon delivery capsules (PCDCs) to improve the bioavailability of glycyrrhizin in solution. They formulated eight types of glycyrrhizin solutions and encapsulated within the

PCDCs. The capsules were evaluated in-vivo in beagle dogs. It was observed that Labrasol, which is a component of self-emulsifying drug delivery systems, strongly improved the bioavailability of glycyrrhizin in colon^[26].

Osmotically controlled systems:

Such systems have a distinct advantage over other systems as they are not dependent upon the physiological conditions of the gastrointestinal tract for drug release but utilize osmotic pressure for controlled drug release from the drug delivery devices. Drug release may depend upon formulations factors like solubility and osmotic pressure of the core component(s), size of the delivery orifice, and nature of the rate-controlling membrane. In the simplest form, such devices contain an inner core containing drug, with or without an osmagent surrounded by a semi-permeable membrane, containing an orifice for controlled drug release. When this system comes into contact with gastrointestinal fluids, water imbibes into the core through the semi-permeable membrane. The drug within the core forms a saturated solution, which is then released in a controlled manner through the orifice depending on the size of the orifice and solubility of the drug. Such systems can be used for drugs with moderate solubility. For drugs with extremes of solubility, push-pull systems can be used. Such systems contain two layers coated with a semi-permeable membrane. The inner compartment consists of polymeric osmotic agents and the outer compartment consists of drug core along with osmagents. When the water is imbibed through the membrane and enters the system, the polymers in the inner compartment swells and forces the drug through the orifice in the membrane. OROS-CT (Alza Corp.) has been developed for targeted delivery to colon and can also be used for systemic action of drugs. It consists of one or even 5-6 push-pull, enteric coated delivery systems within a hard gelatin capsule^[27].

Newer approaches:

Further drug delivery systems have been developed utilizing unique properties of polymers for targeted and controlled delivery in the gastrointestinal tract. For example, Lerner et al developed some gastrointestinal drug delivery systems which comprised of core containing drug and a carrier with swelling properties, which was further coated with water-insoluble or partially soluble material in which a hydrophilic, water insoluble particulate material was embedded. Upon oral administration, when the device comes into contact with the gastrointestinal medium, the particulate matter in the coating swells and form channels which connects the external medium with the core. For water soluble drugs, non-swellable core material was used and for water-insoluble drugs, swellable core material was used. Control of formulation parameters like thickness of coating, amount and particle size distribution of particulate material in the coating, etc. allows for manipulating the location of drug release in the gastrointestinal tract, preferably in the ileum and colon^[25].

Recently, Li et al developed a unique colon-specific drug delivery system referred to as CODESTM. Drug release from this system is triggered by colonic microflora coupled with pH sensitive polymer coatings. The core tablet is coated with an acid soluble, Eudragit E coating, which is further coated with a barrier layer of hydroxypropylmethylcellulose and outer coating of enteric polymer. The barrier layer prevents any interaction between the oppositely charged polymers in the primary and secondary coats. Gamma scintigraphy studies revealed that the CODESTM remained intact in the stomach but the enteric and barrier coats dissolve in the small intestine where the pH is above 6. However, the acid soluble eudragit E coating protects the core in the small intestine, and upon reaching the colon, the polysaccharide in the core dissolves and diffuses through the coating and upon bacterial degradation, the polysaccharide is converted into organic acids. This lowers the pH surrounding the system enough to dissolve the acid soluble coating and subsequent drug release^[28].

Comparative studies have been performed to evaluate the efficacy of the targeted drug delivery systems. In such a study, Takaya et al used three kinds of colon delivery systems for establishing a relationship between in vitro release and in vivo absorption of drug. The three systems used were pressure controlled colon delivery capsules for liquids, time controlled colon delivery capsules for liquids and eudragit S coated tablets for solid preparations. The in vitro dissolution tests for all preparations revealed that the drug release from eudragit S coated tablets delayed drug release the most. However, the colon delivery capsules showed higher systemic availability than the eudragit coated tablets in the in vivo studies^[29].

After so much of research work, the formulations are brought into the market, such that the patients get benefited. Despite of that, the formulations fail some time to stand up to the mark and to deliver expected results.

This is because the performance of the formulations depends on several important factors which must be kept in mind while selecting a particular strategy and developing and evaluating of formulations to target colon. Some of the important factors are discussed below.

Factors affecting therapeutic efficacy of colon targeted systems:

Diverse pH conditions of the gastrointestinal tract:

The colon specific drug delivery systems so designed should be able to prevent drug release in the upper parts of the gastrointestinal tract (stomach and small intestine) and release the drug only in the colon. The human gastrointestinal tract has diverse pH conditions with highly acidic pH 1.2 in the stomach, to alkaline pH in the small intestine 7.2, to near neutral pH in the caecum 6.4 and finally again alkaline pH in the descending colon 7.0^[2]. Further, the pH conditions are not consistent and can vary among different individuals according to age, food habits and disease conditions. For example, significantly higher pH values were found in the feces of patients with colorectal neoplasms^[3]. Marked differences are also found within the same individual on different occasions. Further, the retention time of the dosage form at a specific pH in the gastrointestinal tract is also questionable^[31]. Therefore, the researcher should know, that, which part of the gastrointestinal tract is to be targeted and what may be the pH condition of that particular area affected by disease, food, gender or age. Based on that, the research work should be designed and executed, followed by effective in-vivo evaluation in patients.

Colonic motility:

The colon has a long residence time which is again subject to intra and inter-subject variation. The colonic transit times range within 6-48 hrs but values in excess of 70 hrs have been reported, with men having shorter transit times than women^[31]. The colonic contractile activity can be described by irregular alternation of quiescence, prevalence of non-propagating, segmental contractions and infrequent occurrence of propagated contractions which may be of low amplitude (occurring more than 100 times per day) or of high amplitude (occurring about 4-12 times per day). However under certain disease conditions of the colon, like diarrhea-predominant Irritable Bowel Syndrome, the colonic motility increases, and thus, the residence time of the drug delivery system is lowered, which may result in low therapeutic efficacy^[4]. Again under constipation, the defecation is delayed and thus the residence time in colon is increased. In such cases, the multiple unit dosage forms may be beneficial compared to the single unit dosage forms. Single unit dosage forms may be easily defecated in such cases. The multiple unit dosage forms spread over a wide area throughout the colon and ensure improved therapeutic efficacy. Further, dosage forms with polymers having mucoadhesive properties may be useful.

Volume and availability of colonic fluid:

Volume of the fluids present in the gastrointestinal tract has been measured, with mean values of 118ml in the stomach, 212ml in the small intestine and 187ml in the colon^[35]. However the free fluid volume (water not bound to digesta) is less and exists as fluid pockets, which are irregularly scattered. The free fluid is actually responsible for drug dissolution and absorption. The colonic fluid is highly viscous due to the high absorption capacity of the colon and thus, the availability of drugs to the absorptive membrane is low^[4]. Free fluid in the colon may vary from 1ml to 100ml depending on several factors and thus, the drug dissolution may be seriously affected^[36]. A drug released from the delivery device needs to first dissolve in the surrounding medium, which thereafter is absorbed through the mucosal membrane. If sufficient fluid is not available for drug dissolution, then the absorption of drug may be slow and erratic. Thus systemic absorption of drugs from colonic region may be questionable. However, the multiple unit dosage forms may perform better in this case as they spread over the entire colonic region and require lower amounts of fluid to get dissolved and absorbed.

Colonic microflora:

There are about 400 different species of bacteria present in the colonic fluid including *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, etc. They release several enzymes which are reductive and hydrolytic in nature responsible for the metabolism of drugs in the colon. The primary source of nutrition for these anaerobic bacteria is carbohydrates such as non-starch polysaccharides from the intestinal chime. However,

the composition of colonic bacteria and corresponding enzymes can be influenced by many factors like age, diet, disease, medications and geographic regions^[4]. The bacterial population may also vary according to the disease conditions of the colonic region. For example, in ulcerative colitis and chron's disease patients, more prevalence of *Bacteroides*, *Bifidobacterium* and *E.coli* have been observed.

Further, the colonic conditions in patients may differ from that of normal individuals and the therapeutic management should be monitored likewise to ensure maximum benefit to the patient. In a study conducted by Shobani et al, it was observed that there are differences in the dominant and sub-dominant families of bacteria between normal and colon cancer affected individuals. Among all dominant and subdominant species, *Bacteroides/Prevotella* were higher in cancer individuals than in normal individuals. Further, IL17 immunoreactive cells were expressed more in colonic mucosa of cancer individuals than in normal individuals^[5]. Cummings et al observed in a study that in ulcerative colitis patients there is increased number of organisms but reduced number of protective bacteria like bifidobacteria and lactobacilli. Further, ulcerative colitis patients have increased levels of IgG directed against normal microflora^[6].

Therefore, the researcher requires having knowledge about the microbial population pertaining to different disease conditions of the colon. Accordingly the targeting strategy should be devised and the evaluation methods should also be selected so that the dosage form can be tested on relevant microbial population.

Evaluation Of Colon Targeted Drug Delivery Systems:

The colon targeted drug delivery systems can be evaluated by in vitro and in vivo tests. In vitro tests give a possible idea for the behavior of the systems physiologically. The in vivo tests performed on animals and human subjects exhibit the actual behavior of the systems in the gastrointestinal tract.

In vitro dissolution test:

Using USP dissolution apparatus:

The dissolution test for colon targeted drug delivery systems are performed in USP Dissolution test apparatus II and III in multiple media mimicking the pH conditions of stomach to colon. Generally, the tests are performed in simulated gastric medium (pH 1.2), followed by simulated intestinal medium (pH 7.2) and simulated colonic medium (pH 6.4 with or without rat caecal contents). The duration of testing in each medium are selected in order to simulate the gastrointestinal transit times, which are 2 hours for gastric transit and 3-4 hours for intestinal transit. The maximum mean colonic transit time has been reported to be 33hours for men and 47 hours for women. The gastric transit time varies from one individual to another and may vary from 15minutes to more than 3hours, while the intestinal transit time is fairly constant and on an average a dosage form requires approximately 3 hours to travel through the entire length of small intestine to the colon^[30]. In a study performed by Li et al, the drug release tests performed in dissolution apparatus II (paddle type) and III (reciprocating cylinder) were compared and it was concluded that the reciprocating cylinder method was preferable^[28].

The other factors which may affect the drug release from the delivery device includes the rotation speeds (USP apparatus II) or dip speeds (USP apparatus III), screen sizes of USP apparatus III, viscosity of the medium and volume of the medium.

Dissolution test using caecal contents:

Dissolution tests are performed using caecal contents of rat, rabbit or guineapig for the dosage forms containing polysaccharides in order to mimic colonic conditions. Drug release studies were performed with rat caecal contents of concentration ranging from 2% to 4%.

Dissolution test using fresh human fecal slurries:

Drug release tests have been conducted using fresh human fecal slurries and the fermentation of non-starch polysaccharides has been studied by monitoring the production of short chain fatty acids, acetate, propionate and butyrate vs the total fermentation time. Feces were obtained from healthy human volunteers, homogenized in anaerobic 0.1 M sodium phosphate buffer (pH7.0) to prepare the slurries^[4].

However the availability of fresh fecal samples can be restricted in practice and therefore dissolution studies using several colonic bacteria in culture media have been conducted as an alternative approach to mimic colonic conditions of affected patients. Siepmann et al conducted dissolution studies in culture medium containing mixture of bifidibacteria, bacteroides and E.coli, and found significant increase in drug release, which was comparable with the studies conducted with inoculated fecal slurries^[10].

Dissolution studies using enzymes:

Such type of dissolution studies are mainly done for dosage forms containing polysaccharides as drug carriers which are degraded by colonic bacteria. The colonic bacteria release several hydrolytic and reductive enzymes which are responsible for metabolism of polysaccharides. Therefore, various enzymes (pectinase, dextranase, etc.) are added into the buffer medium for drug release studies in buffer medium to mimic the colonic conditions.

Multi- stage compound culture system:

Three step fermenter- In this fermenter system, three vessels are maintained which mimic the conditions of proximal colon, transverse colon and distal colon with pH values of 5.5, 6.2 and 6.8 respectively. The three reaction vessels are inoculated with 100ml of faecal slurries and samples are collected at regular intervals to determine the enzyme activity, bacterial composition and substrate degradation^[4].

Five-step multi chamber reactor (SHIME)- Five-step multi chamber reactor (SHIME) was developed by Molly et al. In this system, five fermenter vessels are maintained to mimic conditions of the gastrointestinal tract including the duodenum and jejunum (Reactor 1), ileum (Reactor 2), caecum and ascending colon (Reactor 3), transverse colon (Reactor 4) and descending colon (Reactor 5). Reactors 1 and 2 are inoculated with supernatant of a human western diet suspension and Reactors 3, 4 and 5 are inoculated with 50 ml of faecal slurry. A suitable medium was formulated with starch, pectin, xylan, arabinogalactan, glucose, mucin, etc., and the hydrolysis of three prodrugs were tested. The drug release was found to be most pronounced in Reactor 3^[4].

Factors affecting in-vitro dissolution tests:

In-vitro dissolution tests are conducted to give an idea of the actual behavior of the dosage form in the human body. Thus, few factors may be kept in mind while designing the dissolution tests^[31].

1. Fluid volumes:

The in-vitro dissolution tests are performed in 900ml/1000ml of media, where the dosage form may dissolve and disintegrate freely. But the actual free fluid volumes inside the gastrointestinal tract, available for dissolution, are limited.

2. Fluid composition:

We generally employ phosphate, acetate and HCl buffers for dissolution. However, the dissolution rate of enteric coated dosage forms are influenced by buffer capacity and species^[33]. Physiological Krebs's bicarbonate buffer, simulating the ionic composition of ileal fluids, gave better representation of in-vivo disintegration times of enteric coated systems, when compared to phosphate buffers^[34].

3. Surface tension of media:

Surface tensions of the gastric and intestinal fluids affect drug dissolution through wetting and can be mimicked by the addition of bile salts, enzymes and surfactants in the dissolution media^[31]. Fasted state simulated intestinal fluid and fed state simulated intestinal fluid incorporate bile salts (like sodium taurocholate) and phospholipids (like lecithin)^[32]. More and more developments are going on and research is in progress so as to develop appropriate fluid media for dissolution which can mimic the gastrointestinal conditions near to the perfect.

In- vivo experiments:**Organ distribution study:**

Such studies have been conducted for colon specific drug delivery systems in animal models. The dosage forms are administered to the experimental animals and after specified time, they are sacrificed and various parts of the gastrointestinal tract are separated and drug content measured in each part, in order to confirm drug release in the colon^[15].

Oral administration study:

A group of researchers conducted oral administration study, in which a Eudragit S coated tablet for colon delivery was administered orally to adult male beagle dogs and blood samples were collected at regular intervals of time through a 12hr study. The samples were centrifuged, blood plasma was collected and analyzed for drug content. The drug concentration vs time was plotted and the release of drug in various parts of the gastrointestinal tract was assessed from the plot^[29].

Clinical evaluation studies:

Drug release in the colon has been monitored through colonoscopy and intubation, Scintigraphy, radioimaging techniques and high frequency capsules^[30].

Other methods:

Release of drug from chitosan microcores (for colon targeting) was evaluated by inverse dialysis method using 100ml of isotonic pH 7.4 phosphate buffer^[17].

Conclusion:

In this article, we have discussed in details about the advantages of colon targeted drug delivery and the formulation parameters to keep in account based on the physiological conditions of the colon. We have observed that a deep understanding of the physiological conditions of the colon is required for proper targeting and better therapeutic management. We have further discussed the conventional approaches and the novel strategies for colon targeting which promise the best outcomes for effective treatment and management of colonic diseases. Equally important is the development of specific evaluation methods for the delivery devices, which can be used routinely, are easy to follow and inexpensive. It can be rightly said in this regard, that both in-vitro and in-vivo evaluation is necessary for evaluation of the colon targeted systems to get an idea about the efficacy of the systems.

Finally we can conclude that colon targeted drug delivery systems offer several advantages and the recent research in this field have opened up several ways to target the colon effectively and in a controlled manner. However, proper clinical studies should be conducted in order to establish the safety and efficacy of the drug delivery systems which offer high benefits for the patients.

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