Expert Opinion

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Target-specific drug release to the colon

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Background: The ability to deliver drugs to the human colon in a specific manner has become feasible over the years. Targeting pharmaceutical drugs to the colon makes it possible to achieve local or systemic drug delivery to this site. *Objective*: To deliver the compounds in a non-degraded form to the lower part of the gastrointestinal tract, they must first pass through the stomach and the upper part of the intestine before releasing the contents in the colon. *Methods*: This review provides an overview of the various approaches to targeted drug delivery to the colon using different drug delivery systems, their limitations and the future developments in this field. *Results/conclusions*: A microbially controlled system, which is a well-accepted approach, based on natural polymers, has the greatest potential for colonic delivery, particularly in terms of site specificity and safety. However, close attention should be paid to the performance of these products in the heterogeneous environment of the human gastrointestinal tract.

Keywords: biodegradable polymers, colon delivery, colon drug delivery system, colonic microflora

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1. Introduction

Colonic delivery refers to the targeted delivery of drugs into the lower gastrointestinal tract (GIT), which occurs primarily in the large intestine (i.e., colon). The site-specific delivery of drugs to lower parts of the GIT is advantageous for localized treatment of several colonic diseases, mainly inflammatory bowel diseases (Crohn's disease and ulcerative colitis), irritable bowel syndrome, colon cancer and other colon-associated diseases [1,2]. The most critical challenge for such a drug delivery approach is to preserve the formulation during its passage through the stomach and upper part of the small intestine [3-5]. In order to develop a reliable colonic drug delivery system, the transit time of dosage forms through the GIT needs to be thoroughly understood. The transit of orally administered formulations through the GIT is highly variable and depends on various factors [6-9]. For example, factors like disease state of the lumen (diarrhea, diabetes, peptic ulcer, etc.), concomitant administration of other drugs (domperidone, cisapride, metoclopromide, etc.), body posture (vertical or supine) and food type (fat and protein content) can influence the gastric emptying rate. The gastric emptying time of single-unit non-disintegrating dosage forms has been reported to vary from 15 min to over 3 h [10]. In contrast, the small intestinal transit time is fairly constant and varies between 3 – 4 h [11]. The maximum mean colonic transit time in humans is reported to be as high as 33 h in men and 47 h in women [11].

This review aims to collate and understand the novelty and feasibility of different formulation approaches in the development of successful colon-specific drug delivery systems (CoDDS).

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Table 1. Protein and peptide drug candidates for colon-specific drug delivery systems.

Drug	Therapeutic application
A-1 Antitrypsin (aat)	aat deficiency
Amylin	Diabetes and nutrition regulation
Antisense oligonucleotides	Cancer and AIDS
Calcitonin	Paget's disease of bone, hypercalcemia
Cerezyme	Type I Gaucher's disease
Cyclosporine	Immunosuppressants
Desmopressin	Pituitary diabetes insipidus
Epidermal growth factor	Wound healing
Epoeitin	Anaemia-associated chronic renal failure
Etanercept	Rheumatoid arthritis
Filgrastim	Neutropenia
Glucagon	Chronic intractable hypoglycemia
Glucocerebrosidase	Gaucher's disease
Gonadorelins	Endometriosis, infertility
Hirudin	Fibrinolytic
Insulin	Glucose regulation
Interferons	Prophylaxis of hepatitis, malignancy
Leuprolide	Infertility, prostate carcinoma
Molgramostim	Neutropenia
Octreotide	Pancreatitis, acromegaly
Proinsulin	Glucose regulation
Proleukin	Carcinoma
Protirelin	Endometriosis, infertility
Pulmozyme	Cystic fibrosis
Salcatonin	Paget's disease of bone, hypercalcemia
Somatropin	Turner's syndrome, dwarfism
Superoxide dismutase	Respiratory disorders
Urofollitin	Infertility
Vasopressin	Pituitary diabetes insipidus

2. Colon drug targeting

The mucus and epithelium membranes represent crucial physical barriers to the uptake of peptides and proteins from the normal healthy intestinal lumen into the mucosa. Variations in these layers during disease may have important implications for drug delivery to affected regions. In addition to alterations in the colonic mucus layer being affected by number of diseases, the morphology of the mucosa may also be influenced. The permeability of the intestinal mucosa is increased in most patients with Crohn's disease and in 10 – 20% of their clinically healthy relatives. Permeability is also increased in individuals with celiac disease, trauma, burns and those administered with non-steroidal anti-inflammatory drugs. This increased permeability is attributed to the loosening of tight junction [12]. In addition, inflammatory diseases of the bowel, including ulcerative colitis [13] and Crohn's disease [14], are also associated with epithelium inflammation and damage. A break in the continuity of the epithelial surface (whether as a result of tight junction relaxation or a break in the epithelium layer) may offer an opportunity for direct uptake of not only chemotherapeutics, but also of pathological agents by the affected tissues. Abnormal epithelium cell development may lead to benign or malignant growths extending from the colonic epithelium [15]. It is interesting to speculate that the protruding nature of these growths (including polyps) may increase their exposure to the lumen contents and offer a unique opportunity for confrontation with orally administered drugs. Age has a significant impact on the health of the colon and consequently the requirement for therapy. The incidence of colorectal cancer increases with age [15]. Motility disorders of the GIT (such as inflammatory bowel disease [IBD]) also increase with age. It has been proposed that age-associated changes in the levels of neuroendocrine peptides (regulators of GI secretion, absorption, motility, cell proliferation, local immune defense and blood flow) may affect the health status of the colon [16]. From the perspective of therapy, the preferential distribution of non-infective diseases of the colon is particularly important. With the exception of benign colonic carcinoma and Crohn's disease (a disease not restricted to the large intestine [17]), non-infective diseases predominate in the left side of the descending colon and in the sigmoid/rectum. Diseases that dominate this region include large bowel cancer [14], diverticular disease [18], irritable bowel syndrome (IBS) [19] and ulcerative colitis [17]. This preferential distribution is an important consideration for drug targeting strategies. Access to the descending colon would be expected to be more direct through the rectum than through the mouth. However, the ease of oral drug consumption offers a more attractive form of administration than any other alternatives.

Drugs that exhibit good absorption through the colonic wall may be highly beneficial if targeted to the colon. Constant rate drug inputs and improved pharmacokinetic profiles may be witnessed. The colonic environment is free from endogenous digestive enzymes and allows a large residence time. It also does not facilitate any mixing actions, thus making the site suitable for absorption [20]. **Tables 1** and **2** represent some of the peptide and non-peptide drug candidates, respectively, that are absorbed from the colon [20-23].

The advantages of colonic delivery of peptide and protein drugs include:

- low metabolic activity;
- longer residence time;

Table 2. Non-peptide drug candidates for colon-specific drug delivery systems.

Drug	Therapeutic application
5-Fluorouracil	Anticancer
Bleomycin	Anticancer
Bromopheniramine	Antihistaminic
Budesonide	Anti-inflammatory
Dexamethasone	Anti-inflammatory, anti-allergic
Diclofenac sodium	Anti-inflammatory, analgesic
Doxorubicin	Anticancer antibiotic
Ibuprofen	Anti-inflammatory, analgesic
Isosorbide	Anti-anginal
Mesalamine	Anti-inflammatory
Metoprolol	Antihypertensive
Nicotine	CNS stimulant
Nifedipine	Calcium channel blocker
Nimustine	Anticancer
Oxprenolol	Antihypertensive
Prednisolone	Anti-allergic, immunosuppressant
Pseudoephedrine	Bronchodilator
Theophylline	Anti-asthmatic

- responsiveness to absorption enhancers;
- good targeting opportunities due to the presence of colonic bacterial enzymes;
- improved absorption for ionized drugs due to transmucosal and membrane potential differences;
- scope for solvent drag due to bulk water absorption in this region.

If the problems of limited bioavailability and successful targeting to the colon are resolved, the colon could become a potential site for proteins and peptides.

3. Metabolic activity of the colonic microflora

The human colon possesses a dynamic and ecologically diverse environment containing over 400 distinct species of bacteria with a population of 10^{11} – 10^{12} CFU/ml [24-26], with bacteroides, bifidobacterium, eubacterium, lactobacillus, etc., greatly outnumbering other species. For example, it was reported that bacteroides, bifidobacterium and eubacterium could constitute as much as over 60% of the total cultivable flora [26]. These bacteria produce a wide spectrum of enzymes that, being reductive and hydrolytic in nature, are actively involved in many processes in the colon, such as carbohydrate and protein fermentation, bile acid and steroid transformation, metabolism of xenobiotic substances, as well as the activation and destruction of potential mutagenic metabolites. Nitroreductase, azoreductase, *N*-oxide and sulfoxide reductase are the most extensively investigated reductive enzymes, while glucosidase and glucuronidase are the most extensively studied hydrolytic enzymes. These intestinal enzymes are used to trigger drug release in various parts of the GIT and are subsequently used to degrade coatings/matrices, as well as to break bonds between an inert carrier and an active agent (i.e., the release of a drug from a prodrug). A number of delivery systems rely on hydrolysis of glycosides or polysaccharides to control drug release in various segments of the GIT. In general, the types and activities of bacterial glycosidases are unchanged in ulcerative colitis relative to those in healthy volunteers [27-29]. However, in Crohn's disease patients, differences have been noted both in terms of the concentration of microbes and their enzyme activity. In general, glycosidase activity in Crohn's disease patients is reduced as compared to healthy subjects [30-33].

Apart from polysaccharide hydrolysis, colonic microflora is responsible for numerous metabolic reactions via a wide spectrum of enzymes [34]. In general, it can be said that reduction and hydrolysis are the predominating processes in the lumen of the colon. It is noteworthy that the term 'enzymatic azo reduction' is controversial since it is now clear that electron carriers (redox mediators), such as benzyl viologen and flavin mononucleotide, act as electron shuttles between the intracellular enzyme and the substrate. Lloyd *et al.* [35] suggested that colon-specific drug delivery is a valid approach not because a particular organism possessing a specific azo-reductase exists in the colon, but because low molecular weight electron mediators such as nicotinamide adenine dinucleotide phosphate (NADPH) are present and able to diffuse throughout a swollen polymeric matrix.

4. General considerations for the design of colonic formulations

Formulations for colonic delivery are, in general, delayed release dosage forms that may be designed either to provide a 'burst release' or a sustained/prolonged release once they reach the colon. The proper selection of a formulation approach is dependent upon several important factors, such as: i) the pathology and pattern of the disease, especially the affected parts of the lower GIT or pathophysiological changes in the colon; ii) the physicochemical and biopharmaceutical properties of the drug, such as solubility, stability and permeability at the intended site of delivery; and iii) the desired release profile of the active ingredient. The most common physiological factor considered in the design of delayed release colonic formulations is the pH gradient of the GIT. In normal healthy subjects, there is a progressive increase in luminal pH from the duodenum (pH = 6.6 ± 0.5) to the terminal ileum (pH = 7.5 ± 0.4), a decrease in the cecum (pH = 6.4 ± 0.4), and then a slow rise from the right to the left colon, with a final value of 7.0 ± 0.7 [36]. Some reports suggest that alterations in

gastrointestinal pH profiles may occur in patients with IBD, which should be considered in the development of delayed release formulations [37].

The delivery of drugs to specific sites in the GIT has been studied for several reasons. This article is a review of those drug delivery systems that release the drug from the distal ileum to the sigmoid colon. While excluding some approaches, it is convenient to categorize targeted delivery systems into one of four categories: i) passage of time (temporal control of delivery); ii) pH-based (triggered by a change in local pH as the formulation passes down the GIT; iii) enzyme-based (the enzymes found locally in a region of the gut break down a prodrug or a formulation to release the drug; and iv) pressure-based systems (variations in pressure along the lumen of the GIT are used to trigger drug release).

4.1 Prodrug-based approach

The use of a prodrug approach involves the formation of a covalent linkage between drug and carrier in such a manner that upon oral administration the moiety maintains its integrity in the hostile environment of stomach and small intestine and is converted into the parent drug molecule once it reaches the colon. Site-specific drug delivery through site-specific prodrug activation may be accomplished via the use of some specific property at the target site, such as altered pH or the high activity of certain enzymes relative to the non-target tissues for the prodrug–drug conversion.

As discussed above, numerous enzymes, such as azoreductase, glycosidase, polysaccharidases and cyclodextrinase, are produced by bacterial flora and are successfully exploited for site-specific colon targeting with prodrugs. A number of prodrugs have been developed in the past that are capable of targeting NSAIDs to the larger bowel. The colonic bacteria produce a wide array of glucosidases and polysaccharidases that are capable of hydrolyzing glycosides and polysaccharides. The ability of the microflora to hydrolyze glycosides has therefore formed the basis for the design of steroid prodrugs intended for colon-targeted delivery. Different prodrug approaches such as azo bond prodrug, glycoside conjugate, glucuronide conjugate, cyclodextrin conjugate, dextran conjugate and amino acid conjugates have been tried by different researchers around the globe [38-50]. Exploring the details of these conjugates lies beyond the present review.

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 are 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. Jain *et al.* [51] have discussed exhaustively different enzymes produced by organisms in the human GIT that can attack these prodrugs/biopolymers. Prodrugs using azo linkages are sulfasalazine, ipsalazine, balsalazine and olsalazine. These were developed for the delivery of 5-amino salicylic acid to the colon for localized chemotherapy of inflammatory bowel disease.

4.2 pH-dependent systems: enteric coating

Enteric coatings have traditionally been used for drug substances that cause gastric irritation, produce nausea if released in the stomach, or are destroyed by acid or gastric enzymes [52]. The principle by which enteric coating polymers act is that their solubility is highly pH dependent – the polymers being insoluble in gastric acid but soluble in intestinal fluid. To ensure gastric resistance, a coating should be impermeable until at least pH 5. With some polymers that dissolve at relatively high pH values (pH 8), concern arises as to whether the coating will dissolve promptly at the target site to provide an adequate opportunity for drug absorption. The first reported use of enteric coating is credited to Unna in 1884, who introduced a medication based on keratin-coated pills [53]. The range of materials used to produce enteric coatings has increased greatly since then, with polymers of natural or synthetic origin being the most popular and effective. These long-chain molecules characteristically display acidic or acidic ester groups, which provide the pH sensitivity necessary for enteric coating activity.

In contrast to conventional enteric-coated formulations, colonic formulations are designed to deliver drugs to the distal (terminal) ileum and colon, and use enteric polymers that have a relatively high threshold pH for dissolution. The most commonly used polymers are derivatives of acrylic acid and cellulose. These polymers have the ability to withstand an environment ranging from low pH (~ 1.2) to neutral pH (~ 7.5) for several hours. A detailed list of various enteric polymers is provided in **Table 3**. It is highly desirable for pH-dependent colonic formulations to maintain their physical and chemical integrity during the passage through the stomach and small intestine and reach the large intestine where the coat should disintegrate to release the drug locally [38]. It should, however, be noted that gastrointestinal fluids might pass through the coat while the dosage form transits through the small intestine [51]. This could lead to premature drug release in the upper parts of the GIT and as a result a loss of therapeutic efficacy may occur. One approach to overcome this problem would be to apply higher coating levels of enteric polymers; however, this also allows an influx of gastrointestinal fluids through the coat, and the thicker coats often rupture under the influence of contractile activity in the stomach [54]. In general, the amount of coating required depends upon the solubility characteristics (solubility, dose/solubility ratio) of the drug, the desired release profile and surface area of the formulation, and the composition of the coating solution/dispersion. Coating is one of the simplest formulation technologies

Table 3. Enteric polymers used in the development of modified-release formulations for colonic delivery.

Enteric polymers		Optimum pH for dissolution
Polyvinyl acetate phthalate (PVAP) (Coateric®‡)		5.0
Cellulose acetate trimellitate (CAT)		5.5
Hydroxypropyl methylcellulose phthalate (HPMCP)	HP-50	5.0
	HP-55 and HP-55S	5.5
Hydroxypropyl methylcellulose acetate succinate (HPMCAS)	*LF grade	5.5
	*MF grade	6.0
	*HF grade	6.8
Methacrylic acid copolymer, Type C (Eudragit® L100-55*)		
Methacrylic acid copolymer dispersion (Eudragit® L30D-55‡)		5.5
Methacrylic acid copolymer, Type A (Eudragit® L-100* and Eudragit® L12,5)		6.0
Cellulose acetate phthalate (CAP) (Aquateric®‡)		6.0
Methacrylic acid copolymer, Type B (Eudragitò S-100* and Eudragit® S12,5)		7.0
Eudragitò FS30D‡		7.0
Shellac (MarCoat 125§ and 125N§)		7.0

*Suitable for aqueous dispersion.
‡Available as aqueous dispersion.
§Available as aqueous solution.

available for colon-specific delivery. It also offers significant advantages in terms of cost and ease of manufacture. From a formulation standpoint, coated dosage forms may be either a single-unit system or a multi-particulate system, and each of these may be a single-layer product or a multi-layer product. In single-layered products, the coating may be composed of a single enteric polymer that has a pH-dependent solubility or a mixture of two polymers, one of which is pH-dependent while the other is pH independent. In the case of multi-layer products, the coating is applied in successive layers which could be either based on two enteric polymers that have different pH-dependent solubility profiles, or two polymers, one of which is enteric while the other has a pH-independent solubility but is permeable to intestinal fluids. In either case, the coating can be applied to a wide variety of solid core formulations such as tablets, capsules, mini tablets, pellets or granules. When coated pellets or granules are filled into a gelatin capsule or compressed together with conventional excipients in the form of tablets, the formulation is regarded as a multi-particulate dosage form. The tablets or capsules containing coated pellets or granules can be further coated with a suitable enteric polymer which may be the same as or different than that used for the coating of pellets or granules.

The enteric coating of orally administered dosage forms is an effective method of modifying and obtaining control of drug delivery to the small intestine [55]. Some groups have concluded that the change in luminal pH may not be used reliably and routinely as a mechanism to deliver drugs specifically to the colon [56]. Nevertheless, a number of colonic delivery systems have been designed to exploit such a pH-triggered release. The effectiveness of mesalazine in the treatment of IBD is attributed mainly to a topical action on the intestinal mucosa. Because mesalazine is readily absorbed from the small intestine, metabolized, and excreted in the urine, delayed release enteric coated tablet preparations have been developed to prevent release until the drug has reached the terminal ileum and colon. Several colon-targeted formulations of mesalazine have been successfully commercialized in US and European markets. A few of the commercialized products are listed in **Table 4** [57].

4.3 Timed release/delayed release dosage forms

This approach is based on the principle of delaying the release of the drug until it enters into the colon. Although gastric emptying tends to be highly variable, the small intestinal transit time is relatively constant (or little variation can be observed). The strategy in designing timed release systems is to resist the acidic environment of the stomach and to undergo a predetermined lag time, after which release of the drug takes place. The lag time in this case is the time required by the system to transit from the mouth to colon.

The first formulation to be introduced based on this principle was Pulsincap® (**Figure 1**) [58]. It is similar in appearance to a hard gelatin capsule; the main body is made water insoluble (exposing the body to formaldehyde vapour which may be produced by the addition of trioxymethylene tablets or potassium permanganate to formalin, or any other method). The contents are contained within a body by a hydrogel plug, which is covered by a water soluble cap. The whole unit is coated with an enteric polymer to avoid the problem of variable gastric emptying. When the capsule enters the small intestine the enteric coating dissolves and the hydrogel plug starts to swell; the amount of hydrogel is adjusted so that it pops out only after the stipulated period of time to release the drug contents. The viability of such a system in human volunteers has been confirmed on the basis of evaluation studies [59,60].

Timed release, based on a constant small intestinal transit time, has been used to design a delayed release osmotic dosage form for the delivery to the colon, Oros CT [61]. The Oros CT osmotic therapeutic system can be a single osmotic

Table 4. Marketed drug products for the treatment of inflammatory bowel disease.

Drug	Trade name	Formulation	Dose
Sulfasalazine	Azulfidine Salazopyrin	5-ASA linked to sulfapyridine	500-mg tablet; 1 – 2 g/day
Olsalazine	Dipentum	5-ASA dimer	250-mg capsules and 500 mg tablets; 1 g/day
Mesalamine	Asacol	Eudragit-S coated tablets (dissolves at pH 7)	400-mg tablet; 0.8 – 2.4 g/day
Mesalamine	Salofac	Eudragit-L coated tablets (dissolves at pH 6)	250-mg tablet; 1.0 – 4.0 g/day
Mesalamine	Claversal Mesazal Calitoflak	Eudragit-L coated tablets	1.0 – 2.0 g/day
Budesonide	Entocort	Eudragit-L coated beads	9 mg/day

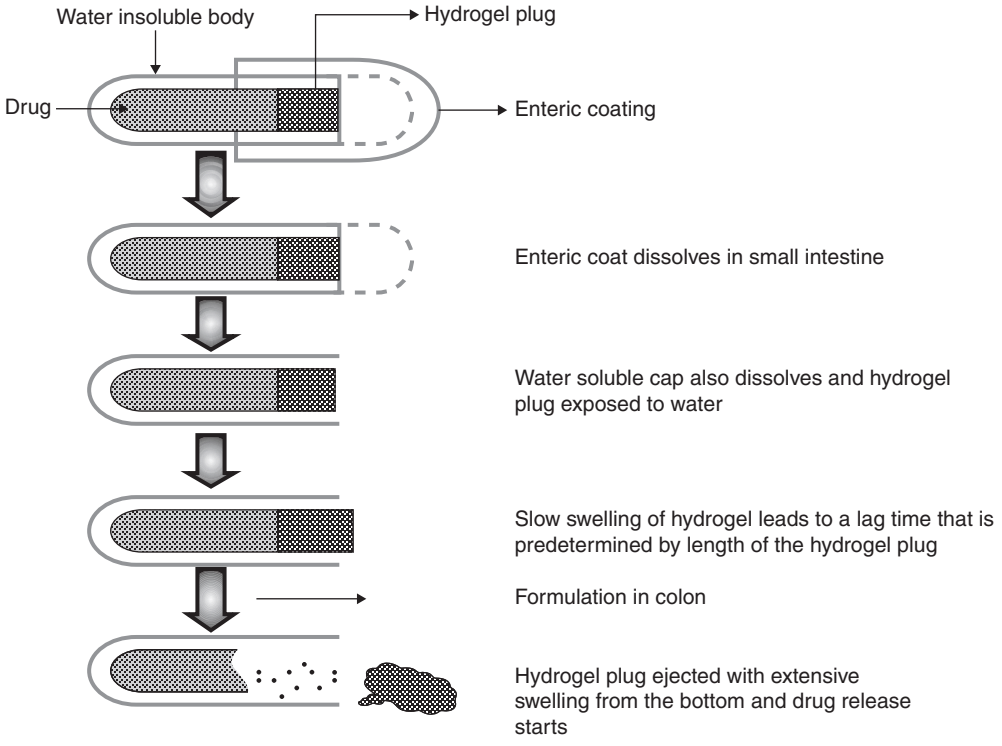

Figure 1. Components and working principle of the Pulsincap time-dependent release system.

unit or can compromise as many as 5 – 6 small push–pull units, 4 mm in diameter, contained within a hard gelatin capsule [62]. Each bilayer push–pull unit contains an osmotic push layer and a drug layer, both surrounded by a semi-permeable membrane. An orifice is drilled through the membrane next to the drug layer. Immediately after the OROS-CT is swallowed, the gelatin capsule containing the push–pull units dissolves. Because of its drug-impermeable enteric coating, each push–pull unit is prevented from absorbing water in the acidic aqueous environment of the stomach and hence no drug is delivered. The individual units are enteric coated to prevent release in the stomach, and the release process is triggered by the change in pH of the intestinal fluid upon gastric emptying. Following triggering, a delay period has been built into the system to coincide with the normal small intestinal residence time: 3 h. Oral administration of 'colon-targeted' osmotic systems containing insulin and permeation enhancers to healthy volunteers has been shown to produce a significant decrease in blood glucose concentration, indicating the absorption of biologically active insulin from the GIT (**Figure 2**) [63].

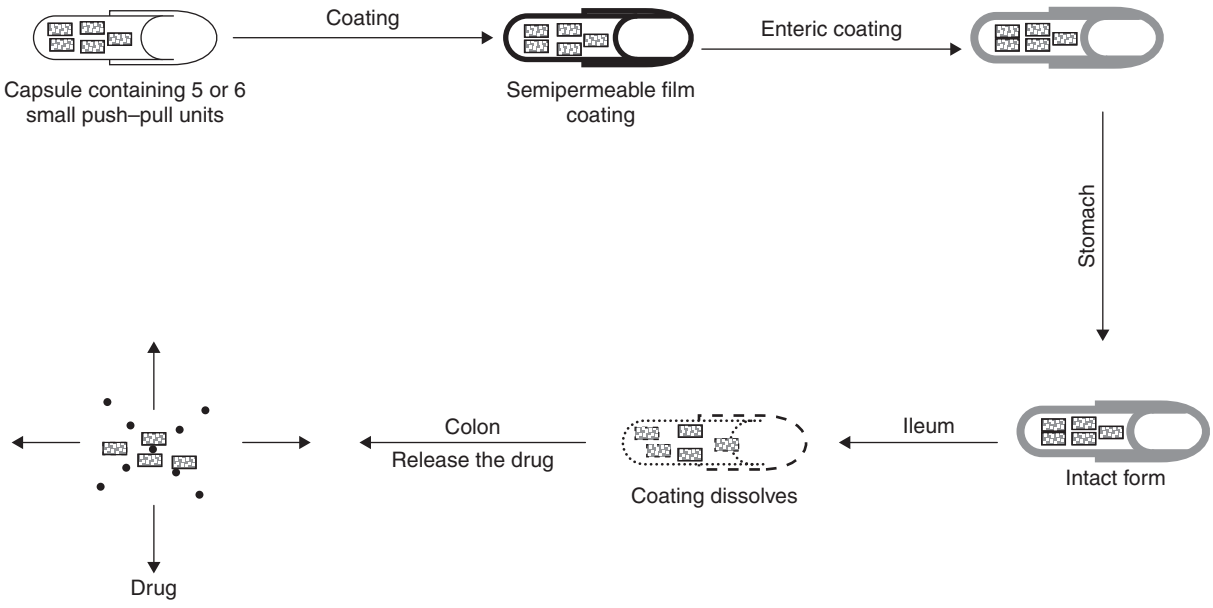

Figure 2. Preparation and release mechanism of the OROS-CT delayed release system.

A multiple-coated oral dosage form consisting of a core coated with three polymeric layers has been developed [64,65]. Gazzaniga *et al.* [65] described a novel oral time-based drug release system for colon-specific delivery. The system is designed to exploit the relatively constant small intestinal transit time of dosage forms and consists of drug-containing cores coated with three polymeric layers. The outer layer dissolves at pH > 5, followed by the intermediate swellable layer, made of an enteric material. The system shows the expected delayed release pattern, as has also been indicated by preliminary *in vivo* studies on rats. Several other drug delivery systems have been developed that rely upon the relatively constant transit time of the small intestine [66-70].

As a new oral drug delivery system for colon targeting, enteric-coated timed-release press-coated tablets (ETP tablets) were developed by coating enteric polymer on timed-release press-coated tablets composed of an outer shell of hydroxypropylcellulose and a core tablet containing diltiazem hydrochloride as a model drug. To clarify whether ETP tablets could be of use in the gastrointestinal tract, ETP tablets with a layer of phenylpropanolamine hydrochloride (PPA) (a marker of gastric emptying) between the enteric coating layer and outer shell were prepared, and were administered to beagle dogs. The gastric emptying time and lag time after gastric emptying was evaluated by determining the times at which PPA and diltiazem hydrochloride first appeared in the plasma [68]. To develop a new colon targeting formulation, which can suppress drug release completely during 12 h in the stomach and release the drug rapidly after a lag time of 3 ± 1 h in the small intestine, the use of press-coated tablets with hydroxypropylmethylcellulose acetate succinate (HPMCAS) in the outer shell was investigated.

In another method, an organic acid (succinic acid) was filled into the body of a hard gelatin capsule as a pH-adjusting agent, together with the drug substance. The joint of the capsule was sealed using an ethanolic solution of ethylcellulose. The capsule was first coated with an acid soluble polymer (Eudragit® E), then with a hydrophilic polymer HPMC, and finally enterically coated with Eudragit® L (**Figure 3**). After ingestion of the capsule, the outermost enteric layer of the coating prevents drug release in the stomach. The enteric layer and hydrophilic layers dissolve quickly after gastric emptying and water starts entering the capsule. When the environmental pH inside the capsule decreases by the dissolution of organic acid, the acid soluble layer dissolves and the enclosed drug is quickly released. Therefore, the onset time of drug release in the intestine can be controlled by the thickness of the acid soluble layer [69].

A delivery system called the Time Clock® has been exploited to release the drug in the colon [71]. It is composed of a solid dosage form coated with a hydrophobic surfactant layer to which a water soluble polymer is added to improve adhesion to the core. The outer layer redisperses in the aqueous environment in a time proportional to the thickness of the film and the core is then available for dispersion. A capsule consisting of ethyl cellulose (EC) was prepared and evaluated for site-specific drug delivery to the colon [72]. It is composed of a low substituted hydroxy propyl cellulose drug container, a capsule body and a capsule made of EC. Water penetrates through the micropores presents at the bottom of capsule and the swelling of the polymer forces the EC cap to disintegrate, thereby releasing the drug.

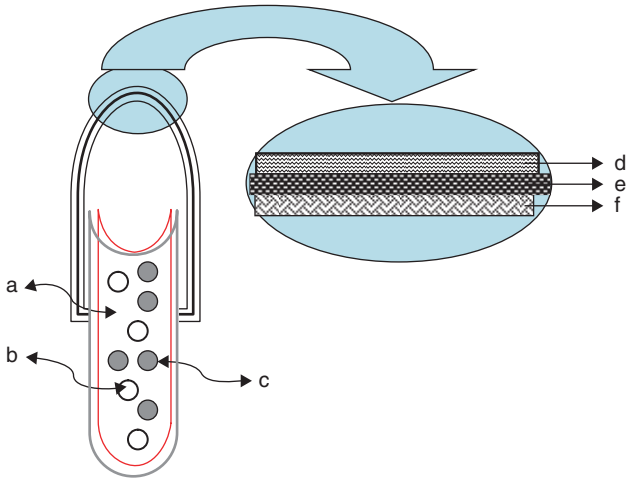

Figure 3. Design of the colon-targeted delivery system. The different components of colon-specific drug delivery systems include: (a) gelatin capsule; (b) active ingredient; (c) organic acid; (d) enteric layer; (e) hydrophilic layer; and (f) acid soluble layer.

Pressure-controlled drug delivery systems have been developed to target the drugs to the colon [73-76]. A pressure-controlled colon delivery capsule (PCDC) made of EC was prepared by coating the inner surface of the gelatin capsule with water-insoluble polymer, EC. By adjusting the coating thickness of the EC membrane to approximately 40 microns, colon delivery of drug was obtained both in beagle dogs and human volunteers. PCDC containing 5-ASA was prepared and administered orally to beagle dogs. After administration, 5-ASA appeared in the systemic circulation at 3 – 5 h, which corresponds to the colon arrival time, confirmed with sulfasalazine [73]. The delivery ability of a PCDC containing caffeine as a test drug was evaluated after oral administration to healthy male human volunteers. The driving force causing PCDC disintegration in the intestinal tract is the physiological luminal pressure, which results from peristalsis. Three kinds of PCDCs with different thickness of water-insoluble polymer membranes were prepared by coating the inner surface of the gelatin capsules with EC.

Evaluation of an oral system (chronotopic) designed to achieve time and/or site-specific release has been reported by Sangalli *et al.* [77]. The system consists of a drug-containing core, coated by a hydrophilic swellable polymer, which is responsible for a lag phase in the onset of release. Another formulation designed to reduce the variability associated with time or pH-dependent drug delivery is based on the CODES® technology [78,79]. This system is more complex than other pH- and time-dependent dosage forms. It relies on the conversion of lactulose to organic acids by colonic bacterial enzymes, as shown and explained in **Figure 4**. Dissolution of acetaminophen from this CODES® formulation was tested in simulated fluids by exposing the system in pH 1.2 for 1 h, then at pH 6.8 for 4 h, followed by at pH 5.0. This dissolution method mimics the conditions anticipated *in vivo* assuming lactulose is released and quickly (and locally) converted to short-chain fatty acids. The dissolution of mesalamine (5-ASA) from a CODES® formulation in beagle dogs under fasted and fed conditions was studied. In this study, increasing the thickness of the underlying Eudragit® R layer was examined. Thicker coatings should limit the rate of availability of lactulose in the colon for degradation. Results obtained were not statistical significant when assessed by $AUC_{(0-14)}$ and C_{max} [79]. Another factor important to the performance of the CODES® system is the amount of lactulose initially loaded into the core. A study designed to assess lactulose loading in the core showed that, like the data with varying coat thickness, no statistically significant differences in $AUC_{(0-14)}$ and C_{max} were found, even though the amount of lactulose used was 38, 58 or 78%. It was found that T_{max} was significantly increased from the formulation prepared with 38% lactulose compared with the 58% and 78% lactulose-loaded formulations. This finding is consistent with the understanding that when less lactulose is released, dissolution of the Eudragit® E coating is slower. No data from a core of 0% lactulose were presented in this study [79].

4.4 Commensal bacteria

The body surface supports the growth of a variety of bacteria and fungi, which are collectively called normal flora or 'normal microbiota'. The normal flora comprise of a permanent population of organisms that vary in both number and kind from one site to another. These microbes are harmless, and for the most part they do not cause disease and are even beneficial. The normal flora can be commensal, when they benefit from the association with the host, whereas the host is not affected. In other words, they enjoy a symbiotic relationship. They may even have a mutualistic association with the host, where they benefit the host while living in the host's body.

Cellular systems like commensal bacteria may offer the means and opportunities for protein delivery through GIT mucosa by a site-specific drug delivery system. In order to provide site-specific protein targeting, a new live vector system, namely recombinant commensal bacteria, has been developed. They have been evolved to colonize a specific niche such as the oral cavity, the gut, the urogenital tract or the rectum. *L. sporogenes* are normal residents of the mouth, colon, vagina and intestine. Genetically engineered live vector systems such as recombinant commensal bacteria, *L. sporogenes* producing or releasing of an enzyme, packed as granules and coated with polymers may be used as model, which may be helpful in lowering the blood substrate level. After disintegration of the granules, the recombinant strain expresses heterologous proteins on their surface or secrete into the colon. These engineered bacteria act as a conserved pathway to express and anchor their surface proteins to the

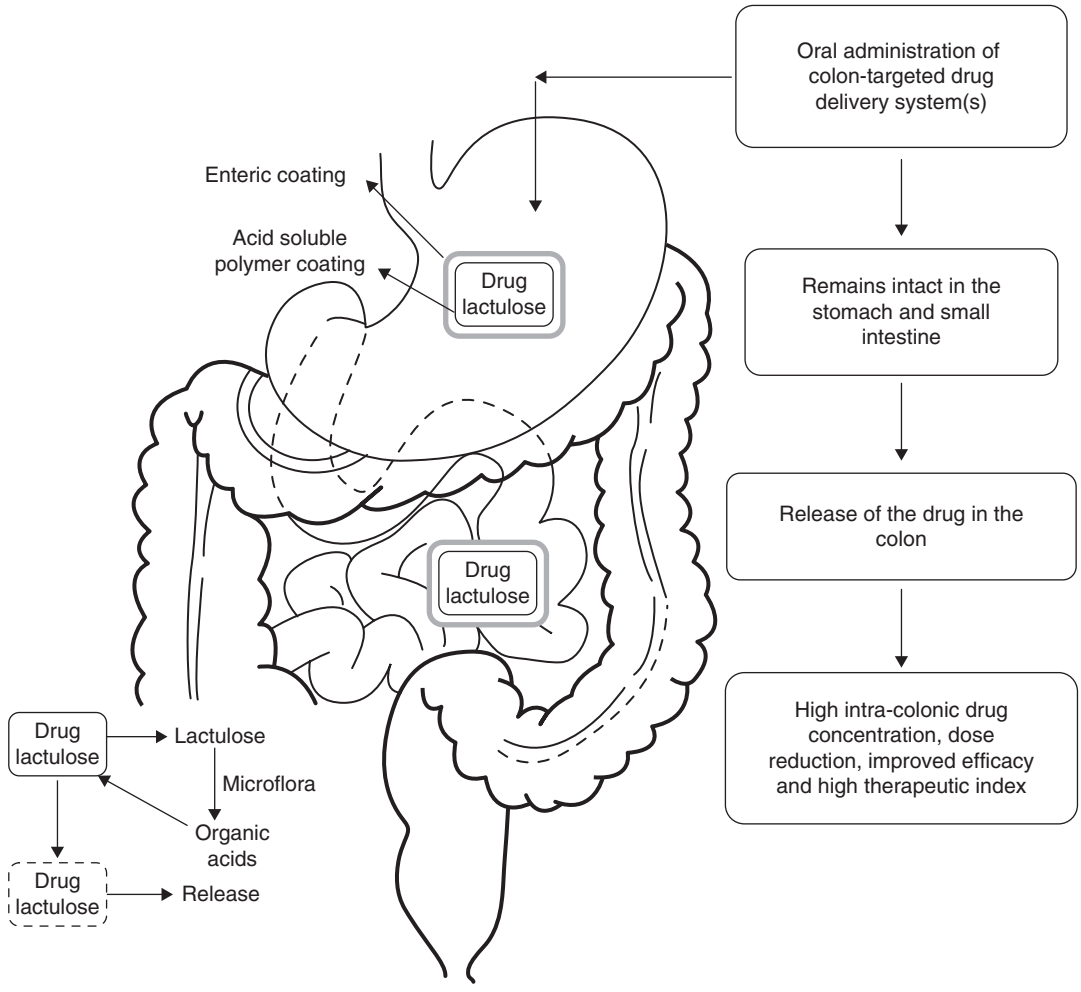

Figure 4. Schematic diagram of the CODES® formulation [78]. The outer coating is composed of a standard enteric polymer such as Eudragit L. Once the unit passes through the pyloris and into the duodenum, this coating dissolves, exposing the undercoating, which is composed of Eudragit® E. This coating will not dissolve in the environment of the small or large intestine. The undercoating permits lactulose to be released into the environment adjacent to the tablet. This disaccharide is metabolized to short-chain fatty acids, which lower the local pH to the point where the Eudragit E dissolves. This final dissolution step exposes the core of the tablet, permitting drug dissolution to occur.

colon and provide prolonged delivery of enzyme. This approach can be used to deliver a recombinant commensal at a specific location where it can colonize, grow and subsequently secrete a biologically active protein to produce a desired pharmacological response at the correct physiological site. Such systems would minimize or eliminate the problems associated with conventional dosage forms, as well as other novel drug delivery systems, and succeed in achieving continuous and prolonged delivery of proteins in the colon. Jain *et al.* [80] developed and characterized engineered commensal bacteria for site-specific targeting of galactokinase and glutamate dehydrogenase. Hope *et al.* [81] discussed the role of bacteria in colorectal cancer from molecular and animal model studies. They focused on some of the mechanisms by which the colonic microbiota drive the progression towards colorectal malignancy, including generation of reactive metabolites and carcinogens, alterations in host carbohydrate expression and induction of chronic mucosal inflammation.

4.5 Redox sensitive polymers

Analogs to azo bond cleavage by intestinal enzymes, novel polymers that hydrolyzed non-enzymatically by enzymatically generated flavins, are being developed for colon targeting. Biodegradation of azo polymers has been extensively studied in the literature [82,83]. It is suggested that both an intracellular enzymatic component and extracellular reduction exist. Under anaerobic conditions, bacterial azo reduction by enzymatically generated reduced flavins where the initial substrate thought to be involved in cellular electron transport requires the presence of NADPH as its electron source. As NADPH is oxidized, the electron mediator

(reduced flavins) acts as an electron shuttle from the NADPH-dependent flavoprotein to the azo compound. Bragger *et al.* [84] carried out investigations into the azo reducing activity, in order to enlighten some factors affecting the bacterial reduction (cleavage) of azo compounds. A common colonic bacterium, *Bacteroides fragilis*, was used as test organism and the reduction of azo dyes amaranth, Orange II, tartrazine and a model azo compound, 4,4′-dihydroxyazobenzene, were studied. It was found that the azo compounds were reduced at different rates and the rate of reduction could be correlated with the redox potential of the azo compounds. 4,4′-dihydroxyazobenzene ($E_{½}$-470 mV) was reduced at the fastest rate of 0.75 mol l^{-1} h^{-1}, amaranth ($E_{½}$-568 mV) at 0.30 mol l^{-1} h^{-1}, Orange II ($E_{½}$-648 mV) at 0.2 mol l^{-1} h^{-1} and tartrazine ($E_{½}$-700 mV) at 0.08 mol l^{-1} h^{-1}. Similar observations were made with another colonic bacterium *Eubacterium limosum*.

Disulfide compounds can also undergo degradation due to the influence of redox potential in the colon [85]. Non-crosslinked redox-sensitive polymers containing an azo and/or a disulfide linkage in the backbone have been synthesised [86]. Radiological studies in dogs have investigated the *in vitro* behavior of new polyurethane systems containing azo bonds [87,88].

4.6 Biodegradable polysaccharide carriers

The polysaccharides naturally occurring in plant (e.g., pectin, guar gum, inulin, locust bean gum, glucomannan, Khaya and Albizia gums), animal (e.g., chitosan, chondroitin sulfate, hyaluronic acid), algae (e.g., alginates), or microbial (e.g., dextran), starch polysaccharides (starch, amylase, cellulose), bacterial (dextran, cyclodextrin, curdlan) and from fungal sources (sceleroglucan) have previously been studied exhaustively [89-92] and will not be discussed further here. These polysaccharides are broken down by the colonic microflora to simple saccharides. Most of the polysaccharide-based delivery systems protect the bioactive from the hostile conditions of the upper GIT. Hydrolysis of the glycosidic linkages on arrival in the colon triggers the release of the entrapped bioactive. The main saccharolytic species responsible for this biodegradation are *Bacteroides* and *Bifidobacteria*. The colon-targeted delivery systems based on these polysaccharide systems with minimal chemical modifications will be discussed in detail.

The use of gastrointestinal microflora as a mechanism of drug release in the colonic region has been of great interest to researchers in recent times. The majority of bacteria are present in the distal gut, although they are distributed throughout the GIT. Endogenous and exogenous substrates, such as carbohydrates and proteins, escape digestion in the upper GIT but are metabolized by the enzymes secreted by colonic bacteria [93]. Sulfasalazine, a prodrug consisting of the active ingredient mesalazine, was the first bacteria-sensitive delivery system designed to deliver the drug to the colon [94]. The use of polysaccharides offers an alternative substrate for the bacterial enzymes present in the colon. Most of the polymers are used in pharmaceutical compositions and are considered to be generally regarded as safe (GRAS) excipients. Pectin alone and in combination with other polymers has been studied for colon-specific drug delivery. Pectin, when used alone, was needed in large quantities to control the release of the drug through the core. A coating composition of a mixture of pectin, chitosan and hydroxypropyl methylcellulose was proven to be very efficient, as the tablets coated with this composition passed intact through the stomach and small intestine and broke in the colon [93,94].

4.7 Targeting of nanoparticles to inflamed areas of the intestinal mucosa

The use of polymeric nanoparticles constituted of poly (lactic acid-co-glycolic acid) (PLGA) for targeted oral drug delivery to the inflamed gut tissue in IBD was examined [95,96]. Such a strategy of local drug delivery would be a distinct improvement compared with existing colon delivery devices for this disease. An experimental colitis was induced by trinitrobenzenesulfonicacid (TNBS) to male Wistar rats. Rolipram, an anti-inflammatory model drug, was incorporated within PLGA nanoparticles, which were administered once a day orally for five consecutive days. A clinical activity score and myeloperoxidase activity were determined to assess the inflammation, whereas an adverse effect index reflected the remaining neurotrophic effect of rolipram resulting from its systemic absorption. All nanoparticle formulations proved to be as efficient as the drug in solution in mitigating the experimental colitis. The clinical activity score and myeloperoxidase activity decreased significantly following the oral administration of rolipram nanoparticles or solution. During the next five days when animals were kept without drug treatment, the drug solution group displayed a strong relapse, whereas the nanoparticle groups continued to show reduced inflammation levels. The rolipram solution group had a high adverse effect index, whereas the rolipram nanoparticle groups proved their potential to retain the drug from systemic absorption, as evidenced by a significantly reduced index. This new delivery system enabled the drug to accumulate in the inflamed tissue with higher efficiency than when given as solution. The nanoparticle deposition in the inflamed tissue should be given particular consideration in the design of new carrier systems for the treatment of inflammatory bowel disease.

5. Engineered capsules for drug absorption studies

Rational and cost-effective development of oral products depends on understanding of the rate and extent of drug absorption from different regions of the GIT. The properties of a drug cannot be used precisely to predict the optimal site of absorption, and often drugs are dropped from the

development pipelines of pharmaceutical companies because of the lack of this understanding. However, despite the clear requirement for human absorption data on both conventional and biotechnology drugs, there is a significant lack of such information in pharmaceutical companies; that is a lack of simple and easy to use technology that can provide such information under non-invasive conditions.

The most popular approach to obtaining information on drug absorption from the GIT has been perfusion or intubation methods [97]. These involve the placing of tubes via the mouth or rectum into the relevant part of the GIT. Once the tube is located at the correct region, a drug solution or suspension is infused into the gut lumen at a predetermined rate. Clearly, these invasive procedures are associated with significant volunteer discomfort, and more importantly the presence of a tube in the intestine can alter the function of the GIT [98]. In particular, intubation has been shown to influence the absorption and secretion balance within the gut, which challenges the pharmaceutical relevance of drug absorption data collected using this approach.

In recent years, there has been a significant growth in the development of engineering-based capsule systems to allow collection of absorption data non-invasively [99,100]. The main emphasis of these technologies has been to permit the operator to control the time and position of the drug release in the gut.

1. High frequency capsule: it is 25 mm in length and 12 mm in diameter, with a drug reservoir of about 1 ml. The location of the high frequency capsule is tracked using X-ray fluoroscopy or gamma scintigraphy. X-ray fluoroscopy for the study of colonic absorption is severely limited because of its high radiation dose [101,102].
2. Gastrotarget® capsule: this gastrotarget telemetric capsule uses carbon dioxide to release 200 µL of the contents of the capsule. An externally detectable dosing indicator signal gives confirmation of *in vivo* release [103].
3. Telemetric capsule: the telemetric capsule weighs 3.5 g and is 39 mm in length. The location of the capsule in the small bowel can be assessed by the investigator using a radio transmitter (and a location detector). The main disadvantage reported with this technology is prolonged gastric retention because of its physical size [104].
4. Intelisite®: the capsule is 10 mm in diameter and 35 mm in length, with a drug reservoir of 0.8 ml in volume. The capsule is based on the use of shape memory alloy (SMA) and heat. The capsule is able to deliver solutions/suspensions and powders. The main delimiting factor in commercializing this product is leakage of reservoir contents before arrival at the relevant intestinal site. Other factors include the failure of successful activation of the capsule if it is located deep inside the body [105].
5. Enterion®: this capsule was developed by Phaeton Research (Nottingham, UK) for targeted drug delivery along the GIT. It is 32 mm long and 9 mm diameter in which up to 1 ml of either powder or liquid formulation can be incorporated. A separate sealed compartment in the capsule can be loaded with a radioactive marker to allow real-time visualization of the capsule location using the imaging technique of gamma scintigraphy [99]. Its seal integrity is good to avoid leakage, offers reliable activation at target site and it shows a good expulsion mechanism for rapid and active expulsion of formulation to any region of GIT [99].

6. *In vitro*/*in vivo* performance criteria

Can we predict the behavior of these systems in the GIT? One of the challenges in the development of such systems is to establish an appropriate *in vitro* dissolution method that can provide reasonable assurance of *in vivo* performance. This is because the rationale behind a colon-targeted drug delivery system is quite diverse. Additional factors that complicate the development of such dissolution testing include inadequate understanding of the colon's hydrodynamics and motility and how they are affected by pathological states.

Conventional US Pharmacopeia (USP) Apparatus I dissolution testing in different buffers is one of the relatively simple and convenient methods routinely used. This method provides essential information primarily on the functionality of the system performance, rather than validity of the design selected. A number of alternative or unconventional approaches have also been reported for *in vitro* performance evaluation of such delivery systems as the modular fermentor, multichamber reactor or simulated human intestinal microbial ecosystems (SHIME) and rotating beads [94]. In addition to the complexity of the methods, other factors such as set up and operating parameters can significantly affect the output of the results.

USP Apparatus 3 (BioDis®) is another recommended method to predict the *in vivo* performance. This offers multiple advantages, such as using a gradient of media to simulate the passage through different sections in the GIT, varying hydrodynamic conditions and residence times in different media to simulate motility patterns and passage times under fasting and fed states.

Scintigraphy and magnetic moment imaging studies are other recent techniques to visualise the *in vivo* targeting properties of such systems. These techniques can provide real time imaging of the dosage form transit in the GIT. Such studies are much more expensive and time-consuming, but they are complementary to the USP Apparatus 3 system as described above and both these methods together can provide a valuable insight into system performance.

6.1 Dissolution using rat caecal contents

To overcome the limitations of conventional dissolution testing, rat caecal contents have been widely used as alternative dissolution medium because of the similarity of human and rodent colonic microflora. For example, the average $\log_{10}$ viable count of Bacteroides and Bifidobacteria,

two numerically predominant polysaccharide degrading bacteria, is 8.0 and 7.0, and 8.0 and 8.2, respectively, in human large intestine and rat caecum [106]. Another advantage of using rat caecal contents is the relatively easy availability of rats. Rat caecal contents were prepared immediately prior to the initiation of the drug release study, due to the anaerobic nature of the caecum. Generally, rats were anaesthetized and the caecum was exteriorized for collecting the contents. The caecal contents were then diluted with phosphate buffered saline (PBS; pH 7) to obtain an appropriate concentration. This step was conducted under a CO_2 or nitrogen atmosphere to maintain the anaerobic condition. The drug release studies were usually carried out in sealed glass vials at 37°C for a defined period of time. Samples were then withdrawn at different intervals to quantify the amount of drug released with an appropriate HPLC method [107-109].

6.2 Dissolution using human fecal slurries

Freshly prepared human feces slurries have been commonly used to investigate the fermentation of non-starch polysaccharides in which the production of short chain fatty acids, acetate, propionate and butyrate was monitored as a function of fermentation time because bacteria consist of approximately 55% of fecal solids [110]. The bacteriology of gut contents from sudden death victims indicated that the fecal bacteria are representative of those occurring in different regions of the large intestine [111]. This method was also adopted for the dissolution testing of colonic drug delivery systems activated by colon microflora, almost exclusively COLAL® (Alizyme Therapeutics Ltd) technology [112].

All the above-mentioned procedures are just the tip of the iceberg and, considering the inter- and intra-subject variability of physiological GIT parameters, further research in this area is crucial in both the design and characterization of the systems.

7. Concluding remarks

The colonic region of the GIT has become an increasingly important site for drug delivery and absorption. This article has described different drug delivery systems that have already been used in the initial approaches for colon-specific drug delivery. Targeted drug delivery would offer considerable therapeutic benefits to patients, in terms of both local and systemic treatment. Systems that rely on gastrointestinal pH, transit times or pressure for drug release are unlikely to function as reliable and effective colon-specific delivery vehicles.

8. Expert opinion

The reasons supporting extensive research in the field of polysaccharides is that they are non-toxic, easy to work with and are mostly FDA approved. One important point is that colon specificity is more likely to be achieved with systems that use natural materials that are degraded by bacterial enzymes of colonic origin. The challenges in the future will be to find a polysaccharide from which one might be able to obtain a non-permeable film or coating and at the same time a high degradability. Polysaccharides with low water solubility have a better capability of retaining a drug, but at the same time degradability will be low. Because the enzymes of the colon are inducible, it may be possible to induce the necessary enzymes by giving the polysaccharide in advance, prior to intake of the drug delivery system or by the sole presence of the drug delivery system. As mentioned above, many bacteria adhere to particulate material and selectively induce enzymes. However, a general enzymatic induction due to the polysaccharide content of the drug delivery device is not likely to occur because the amount of polysaccharide would be very low. It is, however, necessary to obtain more knowledge of the bacterial flora and whether a more or less permanent induction of an enzyme might change the properties and ecosystem of the colonic flora in undesired directions. Moreover, the cost and ease of manufacture of the delivery system are further considerations that will impact on its likely commercialization and, hence, availability to patients. A bacteria-sensitive natural film coating that can be applied to a range of solid oral dosage forms using conventional processing technology would therefore appear to be the delivery system of choice. Finally, there is a need to obtain standardized *in vitro* methods for evaluation of degradation and release, as well as for an evaluation of animal models for two purposes: first, to allow comparison of results from different laboratories; and secondly to allow for a better correlation to the *in vivo* conditions in humans.

The degradation of azo-polymeric carriers represents a most promising strategy for the delivery of drug to the colon. Apart from the toxicity of the azopolymers, various other factors need to be taken into consideration, such as loading capacity of drugs, response of the azo drug delivery systems with respect to change in pH of the GIT, and particularly its suitability and solubility to the pharmaceutically acceptable solvents used in the preparation of azo drug delivery systems. The adjustment of the hydrophilic–lipophilic balance (HLB) of the azo-polymer is also important in controlling the degree of swelling and subsequently in the rate of drug release. At present, the employment of azo-based carriers in clinical trials appears to be inhibited by two interlinked barriers: first, limited data regarding inter- and intra-subject variability of the azo-reduction process impede the design of molecular systems that can be confidently proposed as reliable candidates for such studies; and, secondly, the selected materials need to be subjected to toxicological studies. The importance of the need for toxicological evaluation is appreciated when the carcinogenicity and mutagenicity of some of the azo-dyes evaluated by the food industry are considered. Nevertheless, azo-polymers for colon-specific

drug delivery offer an advantage over their low molecular weight analogs in that they can be designed to degrade to fragments that cannot be absorbed systemically because of their high molecular weight.

By delivering the drug in different physical forms into a specific region of the intestine, the respective contribution of intestinal permeability and/or *in vivo* dissolution to bioavailability is to be assessed. This is the area where one has to understand the human physiology and inter/intra-subject variability. Once this aspect of the human system is understood, the knowledge gained can be applied to focus research on identifying suitable analytical tools to predict the *in vivo* performance of the developed system. Each of these technologies represents a unique system in terms of design but has certain shortcomings, which are often related to degree of site-specificity, toxicity, cost and ease of scale up/manufacturing. A microbially controlled system, which is a well-accepted approach, based on natural polymers, has the greatest potential for colonic delivery, particularly in terms of site specificity and safety. These systems are degraded by colonic bacterial enzymes on reaching the colon and release the drug. With time, new options for achieving highly specific drug targeting to the colon will be available for therapeutic use.

Over the years, there has been a significant increase in the number of drug delivery strategies for site-specific targeting in the GIT tract. However, only in a few instances has close attention has been paid by researchers regarding the performance of these products in the heterogeneous environment of the human GIT. Rational development of future technologies is critically dependent on understanding the variability in gastrointestinal performance.

This article attempted to review the relevance of the human physiology and pathophysiology, along with the current state-of-the-art site-specific drug delivery, hopefully allowing the reader to appreciate the challenge of targeted oral delivery and the available strategies.

Declaration of interest

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