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Review

Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation

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Abstract

The necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. In the past, the primary approaches to obtain colon-specific delivery achieved limited success and included prodrugs, pH- and time-dependent systems, and microflora-activated systems. Precise colon drug delivery requires that the triggering mechanism in the delivery system only respond to the physiological conditions particular to the colon. Hence, continuous efforts have been focused on designing colon-specific delivery systems with improved site specificity and versatile drug release kinetics to accommodate different therapeutic needs. Among the systems developed most recently for colon-specific delivery, four systems were unique in terms of achieving in vivo site specificity, design rationale, and feasibility of the manufacturing process (pressure-controlled colon delivery capsules (PCDCs), CODES™, colonic drug delivery system based on pectin and galactomannan coating, and Azo hydrogels). The focus of this review is to provide detailed descriptions of the four systems, in particular, and in vitro/in vivo evaluation of colon-specific drug delivery systems, in general.

Keywords: Colon-specific drug delivery; Polysaccharides; Colon targeting; Microflora

1. Introduction

The necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. In addition to providing more effective therapy of colon related diseases such as irritable bowel syndrome, inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, colon specific delivery has the potential to address important unmet therapeutic needs including oral delivery of macromolecular drugs. It has been reported that at least 1 million Americans are believed to have IBD with 15 000–30 000 new cases diagnosed annually (DiPirio and Bowden, 1997). Therefore, it appears that targeted drug delivery with an appropriate release pattern could be crucial in providing effective therapy for this chronic disease. The colon is also viewed as the preferred absorption site for oral administration of protein and peptide drugs, because of the relatively low proteolytic enzyme activities in the colon. It has been demonstrated that insulin, cal-

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citonin and vasopressin can be absorbed in that region (Saffran et al., 1986; Antonin et al., 1992). However, the poor intrinsic permeability across colon luminal epithelium of peptide and protein drugs resulted in very low bioavailability following colon-specific delivery (less than 1%). To facilitate the transport of peptide drugs across the intestinal epithelium, the approach of applying absorption enhancers has been proposed (Fix, 1996). Studies indicated that absorption enhancers performed more effectively in the colon than in the upper gastrointestinal (GI) tract (Mrsny, 1992; Leone-Bay et al., 1998).

Because of the distal location of colon in the GI tract, a colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and effect an abrupt onset of drug release upon entry into the colon. This necessitates a triggering element in the system that can respond to physiological changes in the colon. Overall, the physiological changes along the GI tract can be generally characterized as a continuum, with decreases in enzymatic activity, motility, and fluid content and an increase in pH. These gradual changes in physiological parameters are not suitable for triggering elements to effect a sudden and dramatic change in the performance of a delivery system in order to obtain colon-specific delivery. However, the presence of specific bacterial populations in the colon and an apparent transient, small reversal in the otherwise increasing pH gradient are the exceptions that have been extensively explored as triggering components for initiating colon-specific drug release. In general, four primary approaches have been proposed for targeted colon delivery, namely, prodrugs, pH- and time-dependent systems, and microflora-activated systems. A summary of the four approaches, corresponding triggering mechanism and inherent characteristics is described in Table 1. During the past decade, a large number of delivery systems were developed with an intention of colon-targeted drug delivery. However, the majority was based on pH- and time-dependent concepts with limited in vivo evaluation. As explained in Table 1, the similarity in pH between the small intestine and the colon makes pH-dependent systems less reliable. For time-dependent formulations, the location of initial drug release predominantly depends on the transit time of the system in the GI tract. Despite the relative consistency of transit times in small intestine (Davis et al., 1986), the retention times in the stomach are highly variable. That will result in a spread of

Table 1
Summary of colon-specific drug delivery strategies

Design strategy	Drug release triggering-mechanisms	Comments
Prodrugs	Cleavage of the linkage bond between drug and carrier via reduction and hydrolysis by enzymes from colon bacteria. Typical enzymes include azoreductase, glycosidase, glucuronidase	Prodrug is able to achieve site specificity. However, it will be considered as a new chemical entity from regulatory perspective. So far this approach has been primarily constricted to actives related to the treatment of IBD
pH-dependent systems	Combination of polymers with pH-dependent solubility to take advantage of the pH changes along the GI tract	Unpredictable site-specificity of drug release because of inter-/intra subject variation and similarity of pH between small intestine and the colon
Time-dependent systems	The onset of drug release is aligned with positioning the delivery system in the colon by incorporating a time factor simulating the system transit in upper GI tract	Even though the transit times in small intestine are rather consistent, high variation of gastric retention times makes this approach complicated in predicting the accurate location of drug release
Microflora-activated systems	Primarily fermentation of non-starch polysaccharides by colon anaerobic bacteria. The polysaccharides have been incorporated into the delivery system via film-coating and matrix formation	This strategy is highly promising because non-starch polysaccharides can only be degraded in the colon. It should be pointed out that enzymatic degradation of a polysaccharide matrix is a slow process, usually requiring over 12 h for complete degradation

Table 2
In vivo site distribution of initial system disintegration for Pulsincap™ and Time-Clock® systems

Segment of GI tract	Number of subjects	
	Pulsincap™ ($n = 16$)	Time Clock® ($n = 8$)
Small intestine	5	1
Ascending colon	5	1
Transverse colon	6	3
Descending colon	–	3

initial release sites in the distal GI tract from time-dependent systems. Table 2 shows the distribution of initial drug release sites in vivo from two well-characterized time-dependent systems in healthy subjects, Pulsincap® System (Hebden et al., 1999) and Time Clock® System (Steed et al., 1997) with the programmed lag time of 8 and 9.2 h, respectively. Due to the intersubject variation in GI transit times, the onset of initial drug release occurred in the small intestine in some subjects, while in others the formulations passed the ascending colon intact. Additionally, the performance of a time-dependent formulation can be affected significantly by the pathophysiological conditions associated with the GI tract. Accelerated transit through different regions of the colon has been observed in the patients with the irritable bowel syndrome (Vassallo et al., 1992), the carcinoid syndrome and diarrhea (von der Ohe et al., 1993), and the ulcerative colitis (Reddy et al., 1991). Therefore, time-dependent systems are not ideal to deliver drugs colon-specifically for the treatment of colon-related diseases including ulcerative colitis. Furthermore, when designing systems for the treatment of such diseases, it will be desirable that the drug is released in a bolus fashion upon entry into the colon.

Colon microflora is increasingly recognized as a preferable triggering component in the design of colon-specific drug delivery systems since the abrupt increase of the bacteria population and corresponding enzyme activities in the colon represent a non-continuous event independent of GI transit time. As has been well established, the colon contains over 400 distinct species of bacteria having a population of 10^{11}–10^{12} CFU/ml with *Bacteroides*, *Bifidobacterium*, *Eubacterium* and *Lactobacillus* greatly outnumbering other species (Gorbach, 1971). The primary sources of carbon and energy for these bacteria are polysaccharides present in dietary residues and host-produced secretions (Macfarlane and Cummings, 1991). The metabolism of plant polysaccharides by microflora of the large intestine, and especially the fermentation of non-starch polysaccharides, have been extensively investigated (Salayers and Leedle, 1983; Macfarlane and Cummings, 1991). Enzymes responsible for the degradation of polysaccharides include α-L-arabinofuranosidase, β-D-fucosidase, β-D-galactosidase, β-D-glucosidase, β-xylosidase, with the last three enzymes being the most active (Englyst et al., 1987). Because of the presence of colonic microflora and the fermentation of polysaccharides, this strategy could avoid the drawbacks inherent in time- and pH-dependent systems and thereby exhibit greater degree of site specificity. Furthermore, systems exploiting the unique features of the colon will also accomplish greater site-specificity of initial drug release.

Different aspects of colon-specific drug delivery have been previously reviewed (Friend, 1981; Rubinstein, 1995; Kinget et al., 1998). Therefore, the focus of this article is to review (1) several newly developed colon-specific delivery systems that exhibited exclusivity in targeting colon release, (2) the dissolution testing and (3) in vivo evaluation of colon-specific drug delivery systems in general.

2. Newly developed colon-specific drug delivery systems

2.1. Intestinal pressure-controlled colon delivery capsules (PCDCs)

Intestinal pressure-controlled colon delivery capsules (PCDCs), relies on the relatively strong peristaltic waves in the colon that lead to an increased luminal pressure. It consists of a capsular shaped suppositories coated with a water-insoluble polymer, ethyl cellulose (Takaya et al.,

1995; Hu et al., 2000a; Shibata et al., 2001). Once taken orally, PCDCs behave like an ethyl cellulose balloon since the suppository base liquefies at body temperature. In the upper GI tract, PCDCs are not directly subjected to the luminal pressures since sufficient fluid is present in the stomach and small intestine. Due to the reabsorption of water in the colon (Debongnie and Phillips, 1978), the viscosity of luminal content increases. As a result, increased intestinal pressures directly affect the system via colonic peristalsis (high-amplitude propagated contractions). In response to the raised pressure, PCDCs rupture and release the drug load in the colon. However, it should be noted that our understanding of this raised pressure phase is very limited. It was reported that in healthy subjects this pressure can be as high as ~ 110 mmHg with a duration of 14 s (Bassotti and Gaburri, 1988; Rao et al., 2001). But the activity followed the circadian rhythm, with the occurrence of maximum frequency after waking, meals or with defecation (Rao et al., 2001).

Based on limited in vivo evaluation (Hu et al., 1998), it has been demonstrated that the performance of this system appears to be dependent on the capsule size and the thickness of ethyl cellulose coating. Hu et al. used the biomagnetic measurement system (BMS) to estimate the GI transit characteristics of this system in healthy volunteers (Hu et al., 2000b). It was found that the capsule arrived at the ascending colon 4 and 5 h after oral administration in two subjects, while a model drug, caffeine, was first detected in the saliva of the same two subjects 6 and 5 h following oral administration, respectively. This indicated that PCDCs were able to deliver the drug to the colon.

2.2. CODES™ technology

CODES™ is an unique colon-specific drug delivery technology that was designed to avoid the inherent problems associated with pH- or time-dependent systems (Watanabe et al., 1998; Takemura et al., 2000). The design of CODES™ exploited the advantages of certain polysaccharides that are only degraded by bacteria available in the colon. This is coupled with a pH-sensitive polymer coating. Since the degradation of polysaccharides occurred only in the colon, this system exhibited the capability to achieve colon delivery consistently and reliably. As schematically presented in Fig. 1, *one* typical configuration of CODES™ consists of a core tablet coated with three layers of polymer coatings. The first coating (next to the core tablet) is an acid-soluble polymer (in the present case, Eudragit E® was used) and outer coating is enteric with a HPMC barrier layer in between to prevent any possible interactions between the oppositely charged polymers. The core tablet is comprised of the active, one or more polysaccharides and other desirable excipients. The polysaccharides, degradable by enterobacteria to generate organic acid, include mannitol, maltose, stachyose, lactulose, fructooligosaccharide etc. During its transit through the GI tract, CODES™ remains intact in the stomach due to the enteric protection, but the enteric and barrier coating will dissolve in the small intestine, where the pH is above 6. Because Eudragit® E starts to dissolve at $pH \leq 5$, the inner Eudragit® E coating is only slightly permeable and swellable in small intestine. Upon entry into the colon, the polysaccharide inside the core tablet will dissolve and diffuse through the coating. The bacteria will enzymatically degrade the polysaccharide into organic acid. This lowers the pH surrounding the system sufficient to effect the dissolution of the acid-soluble coating and subsequent drug release.

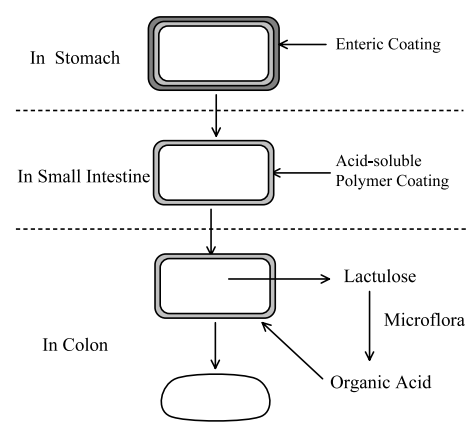

Fig. 1. Schematics of the conceptual design of CODES™ (after Takemura et al., 2000).

2.3. Colonic drug delivery system based on pectin and galactomannan coating

This technology was recently proposed by Lee et al. (1999) and Pai et al. (2000). It consists of a conventional tablet or capsule coated with two specific polysaccharides, namely pectin and galactomannan. By itself, neither pectin nor galactomannan can be used as a drug carrier for colon-specific delivery due to its high water solubility/swelling characteristics. However, the solubility of the coating produced from the mixture of the two polysaccharides was found to predominantly depend on the pH of coating solution. The coating from a pH $\geq$ 7 aqueous solution of pectin and galactomannan was shown to be strong, elastic and insoluble in simulated gastric and intestinal fluids. Accordingly, such coating could protect drug from being released in the upper GI tract. On the other hand, the coating from the identical solution with pH $\leq$ 7 was dissolved readily in the simulated intestinal fluids. Even though the mechanism of this observation remains to be investigated, it was proposed that a complex between the two polysaccharides might be formed at pH $\geq$ 7 due to the hydrogen bonding, hydrophobic force, and the formation of an interjunction zone from conformational changes of polysaccharides at the higher pH.

Results indicated that the bacterial degradability of films produced by this method was still preserved in the colon. It was also demonstrated that the extent of film resistance to hydration and subsequent solublization, the rate of film degradation by enzymes, and the resultant drug release rate depend on the ratio of pectin to galactomannan. Higher percentage of galactomannan results in decreased bacterial degradation in the colon and prolonged duration of negligible drug release in the upper GI tract. The site specificity of drug release was pharmacoscintigraphically confirmed in human subjects (Pai et al., 2000). Compared with the combination of pectin and ethyl cellulose (Wakerly et al., 1996) or amylose and ethyl cellulose (Basit, 2000), this technology might have the advantage of faster degradation in vivo since both pectin and galactomannan are readily degradable by microflora in the colon.

2.4. Azo hydrogels

The synthesis and characterization of a series of novel azo hydrogels for colon-targeted drug delivery have been described (Brondsted and Kopecek, 1991, 1992; Kopecek et al., 1992; Yeh et al., 1995; Ghandehari et al., 1997; Akala et al., 1998). The colon-specificity is achieved due to the presence of pH-sensitive monomers and azo cross-linking agents in the hydrogel structure. During the transit through the GI tract, the swelling capacity of the hydrogels increases as the pH increases, being highest around pH 7.4. Upon arrival in the colon, the hydrogels have reached a degree of swelling that makes the cross-links accessible to the enzymes (azoreductase) or mediators. Subsequently, the hydrogel network is progressively degraded via the cleavage of the cross-links, and the drug entrapped is thus released. The swelling characteristics of the hydrogels can be further controlled by incorporating the hydrolyzable moieties in the hydrogel structure (Akala et al., 1998).

Different synthetic approaches were developed to prepare the hydrogel systems. They can obtained by cross-linking polymerization of *N*-substituted (meth)acrylamides, *N*-*tert*-butylacrylamide and acrylic acid with 4,4′-di(methacryloylamino)azobenzene, 4,4′-di(*N*-methacryloyl-6-aminohexanoylamino) or 3,3′,5,5′-tetrabromo-4,4,4′,4′-tetrakis(methacryloylamino)azobenzene as the cross-linking agents (Brondsted and Kopecek, 1991, 1992). An alternative approach is cross-linking polymeric precursors. In this case, a reactive linear polymeric precursor was first prepared by copolymerization of *N*,*N*-dimethacrylamide, *N*-*tert*-butylacrylaminde, acrylic acid, and *N*-methacryloylglycylglycine *p*-nitrophenyl ester. The precursors were then cross-linked with *N*,*N*′-(ω-aminocaproyl)-4,4′-diaminoazobenzene on the nitrophenyl ester to form the hydrogel structure (Kopecek et al., 1992; Yeh et al., 1994, 1995). The hydrogels were also prepared by polymer–polymer reaction using the same polymeric precursor with the corresponding copolymer containing side chains terminating in NH_2 groups (Ghandehari et al., 1997).

The degradability of these hydrogels has been well characterized both in vitro and in vivo. The

rate of hydrogel degradation was shown to be largely associated with the equilibrium degree of swelling, being inversely proportional to the cross-linking density. It appears that the degradation mechanism of the hydrogel was dependent on to a great extent the synthetic methodology. For hydrogel of the same polymer composition and cross-link structure, these prepared by cross-linking polymerization primarily exhibited a bulk-degradation-like process, while the hydrogel from cross-linking polymeric precursors followed predominantly a surface-erosion process at a low density of cross-linking and became a bulk-degradation-like process when the cross-linking density increased. Hydrogels obtained from polymer–polymer reaction showed the degradation pattern similar to that of the hydrogel from cross-linking polymeric precursors.

To make the hydrogel approach more effective for colon-specific delivery, the hydrogel should be further optimized since preliminary studies in rats indicated that the enzymatic degradation occurred over several days (Brondsted and Kopecek, 1992). An efficient way to incorporate the drug into the hydrogel also needs to be developed.

3. In vitro dissolution testing of colon-specific drug delivery system

Dissolution testing has been an integral component in pharmaceutical research and development of solid dosage forms. It provides decisive information on formulation selection, the critical processing variables, in vitro/in vivo correlation and quality assurance during clinical manufacturing. In order to provide this information, dissolution testing should be conducted in physiochemically and hydrodynamically defined conditions to simulate the environment that the dosage form encounters in the GI tract. Currently, four dissolution apparatus are recommended in the USP to accommodate different actives and dosage forms: basket method, paddle method, Bio-Dis method and flow-through cell method. However, certain constraints associated with USP dissolution methods were recognized, especially in the dissolution evaluation of complex controlled release drug delivery systems for oral application, and modification of USP dissolution methods to evaluate such delivery systems was deemed necessary (Pillay and Fassihi, 1999).

As described above, various mechanisms have been incorporated into colon-specific drug delivery systems. Conventional dissolution testing proposed in USP appears unable to discriminate drug release from systems with different triggering mechanisms. For in vitro evaluation of colon-specific drug delivery systems, the ideal dissolution testing should closely mimic the in vivo conditions with regard to pH, bacteria, types of enzymes, enzymatic activity, fluid volume and mixing intensity. Apparently, such dissolution specifications will be very difficult, if possible at all, to be standardized and validated. Nonetheless, several dissolution methodologies were reported in the literature for the testing of colon-specific drug delivery systems.

3.1. Conventional dissolution testing

Dissolution testing of colon delivery systems with the conventional basket method has usually been conducted in different buffers for different periods of time to simulate the GI tract pH and transit time that the colon-specific delivery system might encounter in vivo (Khan et al., 1999; Takeuchi et al., 2000; Fukui et al., 2000; Rudolph et al., 2001). For example, Takeuchi et al. assessed the dissolution of spray-dried lactose composite particles containing alginate-chitosan complex as a compression coating in pH 1.2 and 6.8 buffer (Takeuchi et al., 2000). Results indicated that such dry-coating showed excellent acid-resistance and prolonged induction periods for drug release. The conventional dissolution method is particularly useful to assess the ability of polymer coating (film coating and dry-coating) to prevent drug release in the stomach or small intestine such as in timed-release system, as well as systems with pH-sensitive polymer coating.

USP Dissolution Apparatus III (reciprocating cylinder) was employed to assess in vitro the performance of guar-based colonic formulations (Wong et al., 1997). Because of the unique setup of dissolution apparatus III (i.e. the dissolution

tubes can be programmed to move along successive rows of vessels), drug release can be evaluated in different medium successively. Wong et al. evaluated several guar-based colonic formulations using apparatus III in simulated gastric fluid (pH 1.2), simulated intestinal fluid (pH 7.5) and simulated colonic fluids containing galactomannanase. As expected, when compared with drug release in simulated gastric and intestinal fluids, results showed that drug release was accelerated in the colonic fluid due to the presence of the galactomannanase that could hydrolyze the guar gum.

Despite the simplicity and convenience, conventional dissolution testing primarily provides essential information on the processing specifications of a colon-specific delivery system rather than on the validity of the system design. For those delivery systems triggered by bacteria in the colon, the conventional dissolution testing appears unlikely to be predictive of in vivo performance. Additional factors that make conventional dissolution testing of colon-specific drug delivery systems less predictive of its in vivo performance are scarcity of fluid and reduced motility in the colon. One function of colon is to absorb water (Debongnie and Phillips, 1978) and thus condense the luminal contents into semisolids. This would influence the drug release from the system and diffusion within luminal contents. Unlike the movement of luminal contents in the stomach and small intestine which is virtually always in the distal direction, it was demonstrated that mixing in proximal colon occurred with reduced motility both laterally and longitudinally even though the mixing can not be defined quantitatively (Wingins and Cummings, 1976). Hence, it is uncertain to what extent the hydrodynamics created by USP dissolution methods reflect the mixing conditions in the colon. Consequently, the drug release determined from the current USP dissolution setting is primarily qualitative in nature and may not be correlated with the in vivo situation.

3.2. Alternative method for evaluation of colon-specific delivery system in vitro

To overcome the limitation of conventional dissolution testing for evaluating the performance of colon-specific delivery systems triggered by colon-specific bacteria, animal caecal contents including rats (Rubinstein et al., 1993; Jung et al., 2000), rabbits (Larsen et al., 1989), pigs (Larsen et al., 1989) have been utilized as alternative dissolution medium. Because of the similarity of human and rodent colonic microflora, predominantly comprising *Bifidobacterium*, *Bacteroides* and *Lactobacillus*, rat caecal contents were more commonly used in the dissolution studies.

Rat caecal contents were usually prepared immediately prior to the initiation of drug release study due to the anaerobic nature of the cecum. Rats were anaesthetized and the cecum was exteriorized for collection of the contents. The caecal contents were diluted with phosphate-buffered saline (PBS, pH 7) to obtain an appropriate concentration for release study. This step was conducted under CO_2 or nitrogen to maintain an anaerobic environment. The drug release studies were generally carried out in sealed glass vials at 37 °C for a defined period of time. Samples were withdrawn at different intervals for analysis (Rubinstein et al., 1992, 1993; Jung et al., 2000; Yang et al., 2001).

Indomethacin release from calcium pectinate tablets was evaluated in 100 ml pH 7 PBS with/without 1.25% w/v rat caecal contents at 37 °C (Rubinstein et al., 1993). Results indicated that indomethacin exhibited greater release in the presence of rat caecal contents when compared with release without rat caecal contents (refer to Fig. 2). In the presence of rat caecal contents, $60.8 \pm 15.7\%$ of drug was released within 24 h in contrast to $4.9 \pm 1.1\%$ drug release in the control medium. This demonstrated that calcium pectinate can be degraded by bacterial enzyme activity. Acetaminophen release from CODES™ was investigated in 20 ml pH 6.8 buffer with or without 10% w/w rat caecal contents (Yang et al., 2001). As illustrated in Fig. 3, in the presence of rat caecal contents, acetaminophen release was essentially completed while no drug release was observed in the medium containing no rat caecal contents within the same time period. This substantiated the design rationale that drug release from CODES™ was triggered by the decrease in pH surrounding the system due to the degradation of lactulose into

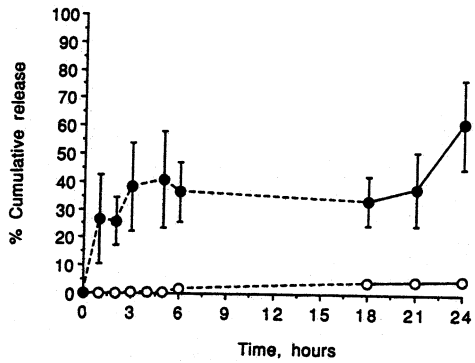

Fig. 2. Percentage cumulative amounts of indomethacin released from CaP tablets in PBS medium, pH 7.0, with (filled circles) and without (open circles) rat cecal content. Data are the mean of three experiments ± S.D. (reproduced from Rubinstein et al., 1993, with kind permission from the publisher).

lactic acid. Dissolution testing in animal caecal contents is especially useful for the 'proof of concept' screening of colon-specific delivery systems based on biodegradable polysaccharides. This method, however, may not generate any meaningful information for colon-specific delivery systems from which drug release is triggered otherwise. It is also unable to evaluate the physical and chemical functionality of the delivery system (such as whether polymer coating is sufficient).

An additional method to evaluate colon-specific drug delivery systems in vitro involves incubation of the delivery system with commonly found colonic bacterium in a modular fermentor under anaerobic conditions. Rubinstein et al. studied indomethacin release from calcium pectinate matrices (Rubinstein et al., 1993), that were incubated in a modular fermentor with *bacteroides ovatus*, a human colonic anaerobe able to hydrolyze pectin. It was found that drug release was higher in comparison to that without *bacteroides ovatus* under the same conditions. A five-step multichamber reactor (simulated human intestinal microbial ecosystem (SHIME)) was also fabricated to simulate the human intestinal microbial ecosystem. In the SHIME, each segment of human GI tract was represented with a reactor. With a medium containing starch, pectin, xylan and arabinogalactan, a reasonable correlation was established between the in vitro/in vivo data with regard to the activity of enzymes commonly found in the human GI tract (Molly et al., 1993). Three polymeric prodrugs of 5-ASA, namely, poly(1-vinyl-2-pyrrolidone-co-maleic anhydride) (PVP-MA), poly[*N*-(2-hydroxyethyl)-DL-aspartamide] (PHEA) and dextran, were evaluated in the SHIME reactor. Little or no hydrolysis of the three prodrugs was observed in the reactors representing stomach and small intestine. However, in the reactor simulating cecum and colon, only dextran-5-ASA exhibited drug release comparable to that of a reference prodrug, sulphasalazine (Schacht et al., 1996).

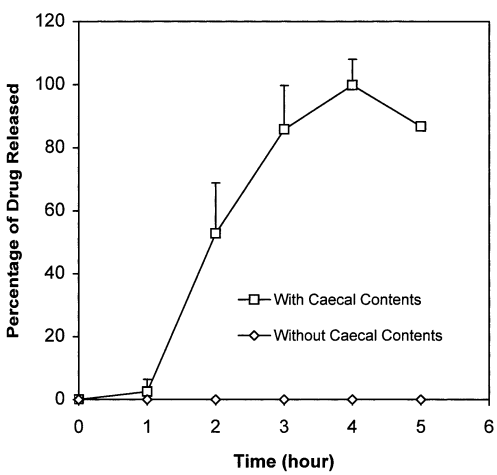

Fig. 3. Comparison of acetaminophen release from CODES™ in pH 6.8 buffer with and without rat caecal contents ($n = 3$, from Yang et al., 2001).

Based on published reports of in vitro evaluation of colon-specific drug delivery systems, it appears that more than one dissolution testing method would be required to fully characterize a delivery system to a greater extent, especially for those systems with a microflora-triggering mechanism. Conventional USP dissolution testing in different buffers can be routinely used to evaluate functionality of the system design, such as different levels of polymer coating, the disintegration time of the core tablet, or dissolution behavior of pH-sensitive polymers. Further dissolution testing using animal caecal contents, bacteria or SHIME would provide some indication of the in vivo performance. It should be noted that the conditions of alternative dissolution methods differed

significantly from each other (i.e. different dissolution volume, different animal caecal contents concentration, etc.). Thus, comparison of colon-specific drug delivery systems in vitro can be problematic. In this respect, application of the SHIME might offer the possibility of standardizing drug release studies. The complexity in setup and operation, however, may prevent SHIME from routine utilization in an industry setting.

4. In vivo evaluation of colon-specific drug delivery systems

As in other controlled release delivery systems, the successful development of a colon-specific drug delivery system is ultimately determined by its ability to achieve colon-specific drug release and thus exert the intended therapeutic effect. When the system design is conceived and prototype formulation with acceptable in vitro characteristics is obtained, in vivo studies are usually conducted to evaluate the site specificity of drug release and to obtain relevant pharmacokinetics information of the delivery system. Although animal models have obvious advantages in assessing colon-specific drug delivery systems, human subjects are increasingly utilized for evaluation of this type of delivery systems with visualization techniques such as γ-scintigraphy imaging.

4.1. Animal studies

Different animals have been used to evaluate the performance of colon-specific drug delivery systems, such as rats (Van den Mooter et al., 1995; Jung et al., 2000; Tozaki et al., 2001), pigs (Friend et al., 1991; Gardner et al., 1996), and dogs (Saffran et al., 1991; Takaya et al., 1995; Shibata et al., 2001; Yang et al., 2001). To closely simulate the human physiological environment of the colon, the selection of an appropriate animal model for evaluating a colon-specific delivery system depends on its triggering mechanism and system design. For instance, guinea pigs have comparable glycosidase and glucuronidase activities in the colon and similar digestive anatomy and physiology to that of human (Hawksworth et al., 1971; Karali, 1995), so they are more suitable in evaluating glucoside and glucuronate conjugated prodrugs intended for colon delivery. Additionally, the carrageenan-induced IBD model in guinea pig is available (Watt and Marcus, 1971). Friend et al. evaluated the therapeutic efficacy of dexamethasone-β-D-glucoside with dexamethasone in guinea pigs with experimentally induced IBD (Friend et al., 1991). Comparable therapeutic outcome was observed with both prodrug and drug as judged by the reduction on ulcer number. However, half dose of the prodrug was needed to achieve the same effect. Even though guinea pig is the preferred animal model to investigate the in vivo performance of certain colon-specific delivery systems, it is difficult to administer the delivery system orally. More often, gastric intubation has to be utilized.

Rats were also used to evaluate colon-specific drug delivery systems based on azo-polymers or prodrugs containing azo bonds because the distribution of azoreductase activity in GI tract is similar between rats and human subjects (Renwick, 1982). Owing to the small size, oral administration of large solid dosage forms (tablets and capsules) is difficult if not impossible. Therefore, capsules were surgically inserted directly to the region of interest in rats (Van den Mooter et al., 1995). Higher plasma concentrations of theophylline were observed when the capsule was inserted in the cecum as compared with the small intestine.

Another animal commonly used to evaluate oral controlled release delivery systems is the dog (Renwick, 1982). Despite the fact that data obtained from dogs does not extrapolate well to human due to the difference of intestinal anatomy and physiology, dogs are increasingly used to evaluate the colon-specific delivery systems (Saffran et al., 1991; Takaya et al., 1995; Shibata et al., 2001; Yang et al., 2001). In these cases, the performance of the delivery system was evaluated indirectly by measuring the plasma concentration profiles of a model drug delivered by the system. However, the location of the delivery system in the GI tract at the onset of drug release can only be estimated by comparing the plasma concentration profiles of drug released from a colon-specific delivery system and the reference dosage.

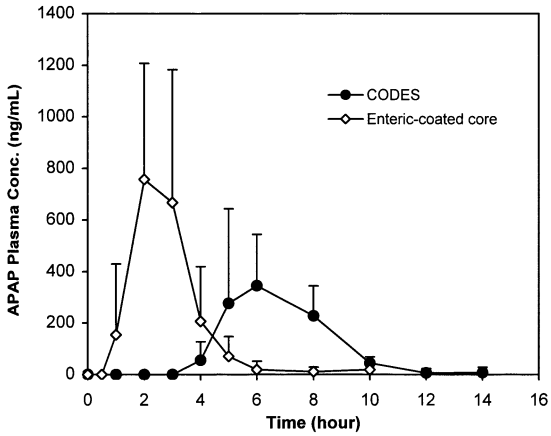

Fig. 4. Percentage of acetaminophen released from CODES™ and enteric-coated core tablets in beagle dogs ($n = 6$, from Yang et al., 2001).

The in vivo performance of CODES™ was evaluated in beagle dogs using acetaminophen as a model drug and lactulose as the matrix-forming excipient in the core tablet (Katsuma, 1999; Yang et al., 2001). Fig. 4 showed the plasma concentration profiles of acetaminophen from CODES™ and enteric coated core tablet. Compared with enteric coated core tablet, the onset of acetaminophen release from CODES™ was delayed more than 3 h. Since the first appearance of acetaminophen from the enteric-coated core tablet was 0.5 h later following oral administration, this suggested that the stomach residence time was very short in this case. The transit time of tablet in the small intestine of beagle dogs has been characterized to be about 2 h (Davis et al., 1993). Therefore, it can be inferred that the onset of drug release from CODES™ took place in the proximal colon of the beagle dogs.

The ability of intestinal PCDCs to obtain colon-specific delivery was also investigated in beagle dogs. Glycyrrhizin, a model drug, did not appear in the systemic circulation until 3.33 ± 1.76 h following oral administration of the system (Shibata et al., 2001). This was consistent with the colon arrival time of 3.5 ± 0.3 h determined with a sulfasalazine study of PCDCs (Hu et al., 1999).

It is well recognized that significant differences exist between human subjects and commonly used laboratory animals in GI tract anatomy and physiology, including GI transit time, pH, distribution of enzyme activity, population of bacteria, etc. Therefore, the data obtained from animal models should be interpreted with caution. In the case of evaluating colon-specific drug delivery systems, the success of a colon-specific delivery system will be primarily decided by the accomplishment of in vivo drug release in the desired location (i.e. colon). Therefore, this event can only be ascertained through visualization.

4.2. γ-Scintigraphy

With growing complexity in the design of novel drug delivery systems (including colon-specific delivery systems) and associated fabrication process, it is critical to understand the in vivo performance of those delivery systems and demonstrate that the system functions in vivo in accordance with the proposed rationale. In most cases, conventional pharmacokinetic evaluation may not generate sufficient information to elucidate the intended rationale of system design. γ-Scintigraphy is an imaging modality, which enables the in vivo performance of drug delivery systems to be visualized under normal physiological conditions in a non-invasive manner. Since first employed to investigate the functionality of tablets and capsules in vivo more than two decades ago (Alpsten et al., 1976; Casey et al., 1976), γ-scintigraphy has become an established technique and extensively used to monitor the performance of novel drug delivery systems within human GI tract. The underlying principles of γ-scintigraphy and its applications in pharmaceutical research and development are available in the literature (Digenis and Sandefer, 1991; Wilding et al., 1991; Newman and Wilding, 1999). Through γ-scintigraphy imaging, the following information regarding the performance of a colon-specific delivery system within human GI tract can be obtained: the location as a function of time, the time and location of both initial and complete system disintegration, the extent of dispersion, the colon arrival time, stomach residence and small intestine transit times. The application of γ-scintigraphy in evaluating colon-specific drug delivery systems was illustrated in the following two examples.

Additional examples can be found elsewhere (Wilding, 1995).

γ-Scintigraphy study of placebo CODES™ was conducted in eight male healthy volunteers to ascertain the time and location of tablet disintegration in the GI tract (Katsuma, 1999; Takemura et al., 2000). Radiolabelled resin (1 MBq of ^{111}In) was incorporated in the core tablet of CODES™, which was then coated with the pH-sensitive polymer coating as described earlier. Gamma camera images were recorded throughout a period of 24 h. The transit and disintegration characteristics of CODES™ in healthy volunteers are presented in Table 3. The average in vivo small intestine transit time was 5.2 h after the system was emptied from the stomach, which is well consistent with the established value of 4 ± 1 h (Davis et al., 1986). The difference between the initial disintegration and colon arrival times is considered as the induction period for acid generation and dissolution of the acid-soluble polymer coating. It can be also observed from Table 3 that the system disintegration in the colon was completed within 60 min. Results further indicated (data not shown) that fed conditions did not adversely affect the CODES™ disintegration profile in vivo even though the gastric residence time was increased.

The in vivo performance of the colonic delivery system based on pectin and galactomannan coating was also evaluated in healthy human subjects with γ-scintigraphy together with conventional pharmacokinetic analysis using nifedipine as a model drug (Pai et al., 2000). Overall, γ-scintigraphic results demonstrated that it took 5.44 h (± 1.77) for the tablets to reach the ascending colon in 92% of 12 subjects. Upon arrival in the ascending colon, approximately additional 1 h was required to initiate the tablet disintegration. Fig. 5 shows the plasma concentration profile of nifedipine from pectin/galactomannan coated tablets and associated in vivo transit and disintegration characteristics. The mean plasma concentration of nifedipine was negligible for more than 5 h post-dose, and then increased rapidly. The pharmacokinetic profile exhibited a good correlation with the scintigraphic results. It should be pointed out that the appearance of nifedipine in the systemic circulation before the average colon arrival time could be primarily attributed to the variation of system colon arrival time between individual subjects.

In essence, γ-scintigraphic evaluation of a colon-specific drug delivery system provides 'proof of concept', i.e. visualization of system disintegration event and ascertainment of disintegration location in the GI tract. Mechanistically, in vivo functioning of colon-specific drug delivery systems involves the interaction between the system and the gut physiology. Thus, it appears that the precise mechanism responsible for the disintegration of a colon-specific drug delivery system

Table 3
Transit and disintegration characteristics of CODES™ under fasted conditions

	Placebo CODES™ (fast)	Reference (fast)
Gastric emptying	0.92 ± 0.48	1.16 ± 0.82
ICJ arrival time	4.51 ± 1.76	4.26 ± 0.10
Colon arrival time	6.12 ± 2.18	7.01 ± 1.94
Initial disintegration	7.11 ± 2.01	10.97 ± 1.62
Complete disintegration	7.87 ± 2.17	13.02 ± 3.37

From Katsuma (1999).

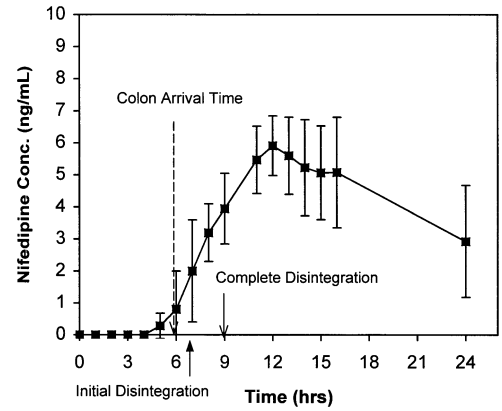

Fig. 5. Nifedipine plasma concentration-time profile from pectin/galactomannan coated tablets and associated in vivo transit and disintegration in human subjects ($n = 12$, from Pai et al., 2000).

can not be determined with γ-scintigraphy imaging. Given the complexity in the functioning of a colon-specific drug delivery system within the GI tract, it is likely that more than one mechanism is involved in the disintegration of the system. For the systems with a microflora degradable film-coating, time-dependent erosion and mechanical failure may play a synergistic role in the disintegration of film-coating.

5. Conclusion

A successful colon drug delivery requires that the triggering mechanism in the delivery system only respond to the physiological conditions particular to the colon. Due to the lack of discontinuity in physiological parameters along the GI tract, few mechanisms can be incorporated into a delivery system to effect colon-specific drug release. So far, four approaches were proposed for colon-specific drug delivery: prodrugs, pH- and time-dependent systems and microflora-activated systems. Of the four approaches, microflora-activated systems appear more promising since the abrupt increase of the bacteria population and associated enzyme activity in the colon represent a non-continuous event independent of GI transit time. Compared with the colon-specific drug delivery systems previously reported, the recently designed systems detailed in this article exhibit the following advantages: commonly used pharmaceutical excipients and feasible processing, site specificity of drug release and versatile drug release kinetics, if so desired.

For in vitro evaluation of a colon-specific drug delivery system, it seems that more than one testing method is necessary to characterize drug release and justify system design rationale. Considering the sophistication of colon-specific drug delivery systems and the uncertainty of current dissolution methods in establishing possible in vitro/in vivo correlation, challenges remain for pharmaceutical scientists to develop and validate a dissolution method that incorporates the physiological features of the colon and yet can be used routinely in an industry setting for the evaluation of colon-specific drug delivery systems. On the other hand, γ-scintigraphy imaging allows the visualization of in vivo functioning of a colon-specific drug delivery system, thereby ascertaining the location of drug release and substantiating the design rationale.

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