Multiparticulate Formulation Approach to Colon Specific Drug Delivery: Current Perspectives

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ABSTRACT

Colon specific drug delivery has gained increased importance not just for the delivery of drugs for the treatment of local diseases associated with the colon but also as potential site for the systemic delivery of therapeutic peptide and proteins. To achieve successful colon targeted drug delivery, a drug needs to be protected from degradation, release and/or absorption in the upper portion of the GI tract and then ensure abrupt or controlled release in the proximal colon. Drug modifications through covalent linkages with carrier or prodrug approach and formulation based approaches can be used for colonic delivery. Report suggests that drug carrier systems larger than 200 μm possess very low gastric transit time due to physiological condition of the bowel in colitis. And for this reason and considering the selective uptake of micron or sub-micron particles by cancerous and inflamed cells/ tissues a multiparticulate approach based on pellets, granules, microsphere or nanoparticle type formulation is expected to have better pharmacological effect in the colon. The review is aimed at understanding recent advancements made in multiparticulate formulation approach for colon specific delivery of medicaments.

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INTRODUCTION

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome but also for the potential it holds for the systemic delivery of proteins and therapeutic peptides. The large intestine, though difficult to reach by peroral delivery, is still deemed to be the ideal site for the delivery of agents to cure the local diseases of the colon (1-2). The most critical challenge in such drug delivery approach is to preserve the formulation during its passage through the stomach and about first six meters of the small intestine (3). In order to develop a reliable colonic drug delivery system, the transit time of dosage forms through the gastrointestinal (GI) tract needs to be understood very well. The transit of perorally administered formulation through the GI tract is highly variable and depends on various factors (4-7). 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. Gastric transit time of single-unit non-disintegrating dosage forms has been reported to vary from 15 min to more than 3 h (8). At the same time, the small intestinal residence time is fairly constant and varies between 3-4 h. The maximum mean colonic transit time in humans is reported to be as high as 33 h in men and 47 h in women (9).

Due to the distal location of the colon in the GI tract, a colon specific drug delivery system should prevent drug release in the stomach and small intestine, and affect an abrupt onset of drug release upon entry into the colon. Such a system can be formulated utilizing some specific conditions existing in the colon in comparison to other parts of the GI tract. Overall, the physiological changes along the GI tract can be generally characterized as a continuum, with decrease in enzymatic activity, motility and fluid content and an increase in pH as we move from esophageal end to the rectum. Another challenge in developing therapeutically effective products for the treatment of colonic pathologies is the impact of disease on the delivery system (10).

For example, the luminal pH of the distal intestine in patients with inflammatory bowel disease (IBD) can be lower than that seen in healthy volunteers. In one study involving six patients with ulcerative colitis, the colonic pH of three patients varied from 5.0 to 7.0, whereas in case of other three subjects very low pH of 2.3, 2.9 and 3.4 were observed (11). Similarly, intestinal transit is found to vary in patients with IBD on account of mucosal inflammation and diarrhea (12). Therefore, prior to the development of any system due consideration must be given to the physiological changes that occur in disease state that might affect the performance of such a system. Inspite of such physiological constraints to drug delivery to colon several colon-targeted formulations have been successfully commercialized in US and European markets. Few of the commercialized products are listed in Table 1.

Several reviews have been published reporting the research that has gone into the development of perorally delivered single unit colon targeted drug delivery systems (13-19). In general four primary approaches have been proposed for colon targeted delivery namely prodrugs, pH dependent system, time dependent systems and colonic micro flora activated systems (16). A brief summary of some of the colon targeted formulations based on the above mentioned conventional approaches is given in Table 2.

Single unit colon targeted drug delivery system may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying (20). Multiparticulate approaches tried for colonic delivery include includes formulations in the form of pellets, granules, microparticles and nanoparticles. The use of multiparticulate drug delivery systems in preference to single unit dosage forms for colon targeting purposes dates back to 1985 when Hardy and co-workers (21) showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to less inter- and intra subject variability. Moreover, multiparticulate systems tend to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption (22-24).

Most commonly studied multiparticulate systems for colon specific drug delivery include pellets, granular matrices, beads, microspheres, and nanoparticles (25-29). This review is aimed at collating and understanding novelty and feasibility of multiparticulate formulation design approach in the development of successful colon specific drug delivery systems.

Table 1. Marketed drug products for the treatment of inflammatory bowel disease

Drug	Trade Name	Formulation	Dose
Mesalamine	Asacol	Eudragit-S coated tablets(dissolves at pH 7)	0.8 -2.4 g/day
Mesalamine	Salofac	Eudragit-L coated tablets(dissolves at pH 6)	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

Table 2. Examples of colon targeted formulations based on conventional techniques.

Technique employed	Polymer (s) used	Drug used	Reference
pH dependent	Eudragit L100 and S100	Mesalazine	31
	Eudragit L100 and S100	NS	32
	Eudragit L100 and S100	Diclofenac sodium and 5-ASA	33
	Eudragit S, Eudragit FS, Eudragit P4135 F	Prednisolone	34
	Eudragit L 30 D-55 and Eudragit FS 30 D	Paracetamol	35
Time dependent	Hydroxy propyl methyl cellulose	Pseudo ephedrine HCl	36
	Hydroxyethyl cellulose,ethyl cellulose, microcrystalline cellulose	Theophylline	37
	Lactose/ behinic acid	Indomethacin	38
	Hydroxy propyl methyl cellulose	NS	39
	Hydroxy propyl methyl cellulose acetate succinate	Diltiazem HCl	40
Bacteria dependent/ Polysaccharide based	Chitosan	Diclofenac sodium	41
	Pectin	Indomethacin	42
	Guar gum	Dexamethasone	43
	Chondroitin sulphate	Indomethacin	44
	Amylose	5-Acetyl salicylic acid	45
	Alginates	5- Acetyl salicylic acid	46

NS, Model drugs used were not specified

DESIGN OF MULTIPARTICULATE DRUG DELIVERY SYSTEMS

The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behaviour due to unit to unit variation, change in gastro-luminal pH and enzyme population. A generally accepted view is that multiparticulate systems perform better *in vivo* than single unit systems, as they spread out throughout the length of the intestine causing less irritation, enjoy a slower transit through the colon and give a more reproducible drug release (20). As in case of single unit dosage forms, for the purpose of designing multiparticulate colon specific drug delivery system, the presence of specific bacterial populations in the colon and an increasing pH gradient have been extensively explored as triggering mechanism in order to initiate colon specific drug release (30). In the following sections, recent innovations in multiparticulate systems for colon specific delivery have been reviewed.

pH- and time- dependent systems

The pH in the terminal ileum and colon (except ascending colon) is higher than in any other region of the GI tract. Thus a dosage form that disintegrates preferentially at high pH levels has good potential for site-specific delivery into this region (32). And in spite of the limitation of change in luminal pH due to disease state, such pH dependent systems are still very commonly investigated for colon targeting.

One of the simplest approaches for designing pH dependent multiparticulate colon specific delivery system is to formulate enteric coated granules. Enteric coating has traditionally been used to prevent drug release in the upper GI tract. Enteric coating polymers are reported to have been used as both binders and as coating materials for granules (48). The influence of incorporating organic acids in granule matrices on drug release has also been studied (49). In one such study, enteric coated tablets of ibuprofen were made from enteric coated granules and citric acid was incorporated in both the granules as well as the matrix (50). It was reported that incorporation of citric acid in both the enteric coated granules as well as the tablet matrix retarded the *in vitro* release and *in vivo* absorption of the

drug because of the prolongation in disintegration time of the core system due to the presence of the acid.

Most commonly used pH-dependent coating polymers for peroral delivery are methacrylic acid copolymers, Eudragit L100 and Eudragit S100, which dissolve at pH 6.0 and 7.0 respectively. The combination of these two polymers in various ratios makes it possible to manipulate drug release within 6.0-7.0 pH range. It has been reported earlier that the use of Eudragit S alone is not suitable for colonic delivery (51). Studies in human volunteers have shown that since the pH drops from 7.0 at terminal ileum to 6.0 of ascending colon, such systems sometimes fail to release the drug (47). In order to overcome this problem, a proper combination of polymers Eudragit S100 and Eudragit L100 ensures that the release of drug from formulation will occur even when the pH value of the GI tract does not reach more than 6.8.

In another study by Akhgari et al (29), the effect of varying the ratios of Eudragit L100 and Eudragit S100 on indomethacin release was evaluated along with the effect of coat thickness using a statistical approach. The drug-binder suspension was sprayed onto non-pareil seeds in a fluidized bed coater. The indomethacin pellets were then further coated with solutions of poly methacrylates again in a fluidized bed coating apparatus. The *in vitro* release studies of the pellets were conducted in media of different pH [1.2 for 2h (stomach), 6.5 for 1hr (proximal small intestine), 6.8 for 2h (lower part of small intestine) and pH 7.2 for 1 hr (terminal ileum)]. It was found that the pellets released no drug at pH 1.2 and 6.5; release was slow at pH 6.8 and was fast at pH 7.2.

Extrusion spheronization technique can be used to prepare uniform-size sturdy pellets when it is not possible to obtain mechanically strong granules by other methods. Krogars et al (52) utilized this technique to produce a commercially viable product of ibuprofen in a matrix base comprising of Eudragit S100 as a binder material, aqueous dispersion of hydroxypropyl methyl cellulose acetate succinate as coating material and citric acid as pH regulating agent. It was found that the excipients had a significant impact on the physical characteristics of the pellets. Eudragit S100 as a pH sensitive matrix base in the pellets increased the pellet size and influenced pellet roundness. Citric acid promoted the pelletization process resulting in a narrower area distribution. However, Eudragit S100 could not cause statistically significant delay in the drug release at lower pH.

In another approach, 5-fluorouracil granular matrices were designed for release of the drug in the descending colon in a controlled fashion for the treatment of colorectal carcinoma. Glyceryl palmitostearate was used as the retardant material to formulate the controlled release matrices. These matrix granules were introduced into enteric coated capsules so as to be carried to and liberated in the ileum (25). The hydroxy propyl methylcellulose capsules were enteric coated with Eudragit FS 30D. It was shown that these capsules disintegrated in the distal portion of small intestine and proximal colon. Capsules of this type could, therefore ensure spatial delivery of drug preferentially in colon without substantial release in the upper GI tract up to the ileum. The matrices were coated by Eudragit S100 and were then covered by a layer of chitosan HCl and loaded inside these capsules. Upon hydration, the capsule shell dissolves and the chitosan layer forms a gel (internal pH of 4.5), which generates an acidic environment around the Eudragit film so that it does not dissolve in the ascending colon. In the ascending colon, the chitosan HCl gel is degraded by the colonic micro flora, thereby exposing the Eudragit film to the colonic environment. But since the ascending colon is weakly acidic where pH is less than 7.0, the film coat still remains intact. However, on arrival in the descending colon where pH is greater than 7, the Eudragit film coat dissolves and the drug is released in a controlled fashion from the matrices. *In vitro* release studies conducted in simulated GI tract environment provided good evidence for the proof of concept that successful targeting to the descending colon could be achieved.

It is accepted that a colonic delivery system which is based only on GI transit time or pH of the GI tract would not be reliable because of the inherent variability of pH and emptying times from the GI tract. In a study by Gupta et al (27), an attempt was made to exploit the relatively constant transit time of 3-4 h of the small intestine and high pH of 7-8 of the distal region of the small intestine. The objective was to combine pH based dissolution characteristics of different Eudragit polymers and that of constant transit time in the small intestine to develop a reliable multiparticulate colonic delivery system. Pellets were chosen for development

because they spread out over a large area and may improve absorption characteristics for large molecules. The drug, 5-aminosalicylic acid (5-ASA) was layered onto non-pareil beads in a conventional coating pan. For the inner coat, the pellets were coated with a combination of Eudragit RL/RS in a fluidized bed coating apparatus. For the outer coat, the above pellets were further coated with Eudragit FS 30D in the fluidized bed processor. Eudragit FS 30D is an-ionic co-polymer of methyl acrylate, methyl methacrylate and methacrylic acid and is pH sensitive and dissolves at pH above 6.5. The advantage of such a system is that it can be easily manufactured on a large scale in a reasonable processing time using conventional powder layering and fluidized bed coating techniques. The delivery system demonstrated its potential for colonic delivery by resisting drug release up to pH 6.5 and the combination of Eudragit RL and RS proved successful for the sustained delivery of 5-ASA at the pH of the colon.

Microbially controlled systems

Amongst all the approaches used for colon targeting, a microbially controlled delivery system is the most appealing as it relies on the unique enzymatic ability of the colonic micro flora and enables a more specific targeting, independent of pH variations along the GI tract. Many natural polysaccharides such as chondroitin sulphate, pectin, dextran, guar gum etc. have been investigated for their potential in designing colon-specific drug delivery (18).

A multiparticulate system consisting of hydrogel beads was formed by chitosan and tri poly phosphate (TPP) for the delivery of protein (26). TPP was used as a counter ion to positively charged chitosan to form gel beads. The beads were loaded with bovine serum albumin (BSA), a protein that is liable to degradation in the upper parts of GI tract. The cross linking of chitosan with TPP resulted in reduced solubility of chitosan, thereby resulting in lesser protein release during upper GI transit. At the same time, the cross-linking and reduced solubility did not affect the degradability by microbial flora in the colon as shown by the in vitro studies where the rat caecal contents were able to attack and degrade the cross-linked chitosan.

The use of polysaccharides for coating purposes has been tried with limited success. Most of the non-starch polysaccharides suffer from the drawback of lacking good film forming properties. Also, they tend to swell in the GI tract and become porous, resulting in the early release of the drug. In a break through study by Milojevic et al (28), the ability of amylose as a film-forming polymer was investigated. Amylose which is the major fraction of starch, possess the ability to form films through gelation. A particular form of coating, comprising of amorphous amylose is resistant to degradation by pancreatic alpha amylase but is capable of degradation by colonic bacterial enzymes. One disadvantage of using amylose in this form is its swelling properties in aqueous media. This could be controlled by incorporating insoluble polymers like, ethyl cellulose and acrylates into the amylose film. Pellets of 5-ASA were prepared by extrusion spheronization and coated with mixed dispersion of amylose and ethyl cellulose in varying ratios using fluidized bed-coating technique. Amylose was also mixed with Eudragit RS/RL 30D aqueous dispersions and the coating was applied to the pellets. It has been found that ethyl cellulose effectively controls the swelling. The amylose-ethyl cellulose coat (in the ratio 1:4 w/w) could resist dissolution in the simulated gastric and small intestinal conditions for over 12 h. However, amylose-Eudragit RS/RL coating system even at high coating thickness did not provide sufficient resistance to degradation in acidic and neutral media. The susceptibility of these films to colonic micro flora was also tested in the batch fermentor where the films were found to degrade and there by exhibit colon specificity.

The ability of amidated low methoxy pectin to form rigid gels with divalent cations has been exploited to produce calcium pectinate gel beads, intended for controlled release delivery of conventional drugs and also as a carrier for colonic delivery of proteins (53). To overcome the problem of high dissolution of pectin in the upper GI tract, pectin has been combined with calcium salts since calcium pectinate (the insoluble salt of pectin) is not degraded by gastric or intestinal enzymes but is capable of degradation by colonic pectinolytic enzymes. Comparative efficacy of zinc pectinate gel microparticles against calcium pectinate gel beads as potential colonic delivery system was explored by El-Gibaly et al (54). The conventional

technique of ionotropic gelation was used to obtain drug loaded beads. The cross linking agent used was calcium chloride and zinc acetate respectively. When the *in vitro* drug release properties were compared, significant retardation was observed in the case of zinc-pectinate microparticles ($t_{50\%}$ of 7.33 h) as against calcium-pectinate based beads ($t_{50\%}$ of 35 min). The observed differences were attributed to the differences in degree of cross linking of the two gel types, which affected the swelling rate of the microparticles, during drug release and consequently the penetration of solvent into the microparticles.

An alternative to crosslinking of soluble polysaccharides to form insoluble salts was suggested by Lorenzo-Lamosa et al (41). Here, the polysaccharide based system was coated with pH sensitive polymers. Chitosan microcores were prepared containing drug (sodium diclofenac) which were coated with acrylic polymers, namely, Eudragit L100 and Eudragit S100 respectively. A multiparticulate system was designed which combined two approaches: pH sensitive delivery and biodegradation in the colonic environment. The system was developed in two steps: first, drug was entrapped within chitosan microcores using spray drying technique and second, drug loaded chitosan microcores were microencapsulated within Eudragit polymers by the oil-in oil solvent evaporation technique. The *in vitro* release behavior of Eudragit microencapsulated chitosan microcores showed no drug release at gastric pH for 3 h. Further, in a pH gradient medium (5.8-7.4), it was observed that no drug release occurred in the pH below the solubility pH of the enteric coating polymers. Upon dissolution of the outer Eudragit coat at appropriate pH the exposed chitosan microcores swelled and formed a gel barrier in alkaline pH through which the drug diffused out. Also, it was postulated that in the colonic region, the chitosan would undergo degradation process, thereby enhancing the release of the entrapped drug.

In a similar approach, a multiparticulate system was proposed comprising of chitosan microspheres coated with Eudragit L100 or S100 for the colonic delivery of metronidazole for the treatment of amoebiasis (55). The drug release was expected to take place after dissolution of the enteric coating in the small intestine and biodegradation of the chitosan in the colon due to presence of polysaccharides in the colonic contents. In order to prevent early loss of drug from microspheres, the chitosan was cross linked with glutaraldehyde. In this study, microspheres were prepared by an emulsion solvent evaporation technique. It was shown that the multiparticulate system successfully prevented the release of drug until it entered into the colon. The release of a higher amount of drug in the presence of rat ceacal contents reveals the susceptibility of chitosan matrix to colonic enzyme digestion.

Microparticulate systems

In the treatment of IBD, sustained release devices like pellets, capsules or tablets have less efficiency due to diarrhea, a symptom of IBD, that enhances their elimination and reduces the total time available for drug release. It has been shown that drug carrier systems with a size larger than 200 µm would be subjected to speedy bowel evacuation due to diarrhea, resulting in a decreased GI transit time and decreased efficiency (56). Therefore, a multiparticulate system in the micron size range could be a useful option in the design of a suitable dosage form for IBD. In the work reported by Lamprecht et al (57), Eudragit P-4135 F, a new pH-sensitive polymer was used to prepare microparticles of tacrolimus, an immunosuppressant drug, for colonic delivery. In a previous study by the same authors, the use of Eudragit P-4135 F in the microencapsulation of 5-fluorouracil for the treatment of colorectal cancer has been reported (58). Eudragit P-4135 F belongs to the pH-sensitive Eudragit group of polyacrylates and possess a dissolution threshold pH slightly above 7.2. This is very useful as ulcerative colitis mainly affects the distal parts of the colon and an early drug loss towards the non-inflamed tissue would be undesirable. Most of the currently employed pH-based colonic drug delivery systems utilize Eudragit S and L which dissolve in the pH range of 6-7 and therefore liberate the drug at the terminal ileum, which may lower the efficiency and at the same time risk adverse effects. Eudragit P-4135 F might prove a useful alternative for systems intended for targeting to the distal colon.

A few studies have indicated the involvement of macrophages and dendritic cells in active IBD (59, 60). Since these immune cells have an important pathophysiological role, they should be

considered in the therapeutic strategy for patients with IBD. It was reported that biodegradable microspheres could be efficiently taken up by macrophages. Therefore, the direct uptake of anti-inflammatory agents loaded microspheres by macrophages would have a superior immunosuppressive effect and be more useful for treatment of patients with IBD. In a study by Hiroshi and coworkers (61), dexamethasone was incorporated into poly (DL-lactic acid) microspheres and administered to mice induced with experimental colitis. It was found that serum dexamethasone levels were not increased after oral administration of dexamethasone microspheres but at the same time the microspheres facilitated mucosal repair of experimental colitis. This strategy could be ideal for the treatment of IBD where local action in colon is needed without systemic drug burden.

Microparticulates in the delivery of peptides

The colon has always attracted attention as a potential site for the systemic absorption of peptide drugs on account of its lower proteolytic enzyme activity compared to the upper GI tract (62, 63). In this context, several research groups have attempted to develop oral delivery systems for insulin (64-66). Rubinstein et al have developed a system consisting of insulin encapsulated by polyacrylates wherein the coating was meant to dissolve only in the colon (67). A terpolymer of styrene and hydroxyethyl methacrylate cross-linked with a difunctional azo-compound has also been reported for the delivery of insulin (68). The system depends on cleavage of azo bond by colonic microflora resulting in degradation of polymer and release of insulin. Mathiowitz et al developed insulin containing polyanhydride microspheres which were shown to adhere to the walls of the small intestine and release the insulin upon degradation of the polymeric carrier (69). This ensured protection of insulin from degradation in upper portion of the gastro-intestinal tract and release into distal portion of small intestine and proximal colon for systemic absorption. In one study, pH responsive poly (methacrylic-*g*-ethylene glycol) hydrogels were investigated as oral delivery vehicles for insulin (70). Insulin was loaded into polymeric microspheres and administered orally to healthy and diabetic Wistar rats. The gel particles did not swell in the acidic environment of the stomach thereby protecting insulin from proteolytic degradation. However, once inside the basic and neutral environment of the small intestine, the gels rapidly swelled and dissociated releasing the entrapped insulin. Within 2 h of administration, strong hypoglycemic effects could be observed in both healthy and diabetic rats.

More recently, a microcapsule formulation for the peroral colon-specific delivery of water–soluble macromolecules like peptide drugs has been developed (71). Since peptides are unstable at high temperatures, a polymer was designed that possessed good film formability at low temperatures and a desired permeability to allow a time dependent delayed release profile. A series of aqueous colloidal terpolymers of ethylacrylate/ methyl methacrylate/ 2- hydroxyl ethyl methacrylate (poly (EA/MME/ HEMA) were synthesized by emulsion polymerization technique(s) and their coating performance and solute permeability as a microcapsule membrane investigated. It was found that these polymers exhibited delayed release profiles which were characterized by a long lag time and subsequent rapid release of the entrapped moiety (lactose in this case). This finding led the authors to explore the possible application of these microcapsules for the colon-specific delivery of peptide drugs. Since the method employed fluidized bed based spray-coating, the stability of peptides during the coating operation became a critical issue. In order to secure the thermal stability of peptides, the temperatures in the whole preparation process were controlled at 40°C. The effect of different relative polymer ratios on *in vitro* release was studied (71). Fluorescence labeled dextran (molecular weight 9500) was used as a model drug as it simulates the release of macromolecular compounds such as peptides. It was shown through the study that the coating films delayed the release of the water-soluble compound by 5 h. The lag time could be adjusted by altering coating thickness, thereby allowing site-specific targeting of entrapped drug.

Nanoparticulate systems

Nanoparticle size colloidal carriers composed of natural or synthetic polymers have also been

investigated for colon targeting. Orally administered nanoparticles serve as carriers for different types of drugs and have been shown to enhance their solubility, permeability and bioavailability (72). Nanoparticles have also been investigated for the delivery of protein and peptide drugs (73).

For colonic pathologies, it was shown that nanoparticles tend to accumulate at the site of inflammation in IBD. This is because in case of colitis, a strong cellular immune response occurs in the inflamed regions due to increased presence of neutrophils, Natural Killer cells, macrophages and so on. It has been reported that microspheres and nanoparticles could be efficiently taken up by these macrophages (74). This results in accumulation of the particulate carrier system resulting in prolonged residence time in the desired area. A study by Lamprecht et al (75) proved an increased nanoparticle deposition in the inflamed tissue of the colon compared to the healthy control.

However, an important area of concern is to prevent loss of nanoparticle in the early transit through GI tract in order to optimize therapeutic efficacy (57). Moreover, particle uptake by Payer's patches and/or enzymatic degradation may cause the release of entrapped drug leading to systemic drug absorption and side effects. In order to overcome this problem, drug loaded nanoparticles were entrapped into pH sensitive microspheres, which serve to deliver the incorporated nanoparticle to their site of action, thereby preventing an early drug leakage. The use of Eudragit P-4135F prevented drug release in the upper GI tract and during intestinal passage and permitted selective drug delivery in the colon.

The use of nanoparticles for bioadhesion purposes have also been investigated (78). Nanoparticles have a large specific surface, which is indicative of high interactive potential with biological surfaces. Since the interaction is of non-specific nature, bioadhesion can be induced by binding nanoparticles with different molecules. For covalent attachment, the nanoparticle surface has to show free functional groups, such as carboxylic or amine residues. In one study, nanoparticles were prepared from gliadin protein isolate from wheat gluten, as these can be readily used for binding ligands to their surface. The gliadin nanoparticles were conjugated with lectins (glycoproteins of non-immune origin which provide specific bioadhesion), fluorescently labeled and evaluated for bioadhesive potential on isolated intestinal segments. It was shown that gliadin nanoparticles have a high capacity of non-specific interaction with intestine and the binding of lectin provided greater specificity for colonic mucosa.

In vivo BEHAVIOR OF MULTIPARTICULATE SYSTEMS

Gamma scintigraphy based imaging technology has immensely helped in generating good evidence of the actual *in vivo* behavior of colon targeted dosage forms (77, 78). Various parameters like GI transit time, residence time in small intestine, colon arrival time, and residence time in colon constitute vital information for *in vivo* evaluation and establishing *in vitro-in vivo* correlation of colon targeted dosage forms (79, 80).

In one such study, the GI transit of a multiparticulate dosage form in the form of pellets and a non-disintegrating tablet of metoprolol were studied by Abrahamsson and co workers (81). The two formulations were simultaneously administered with breakfast to eight healthy male human subjects. A statically significant difference was reported between the mean gastric emptying time for the pellets (3.6h) and that for the tablet (9.6 h). However, the mean transit through the small intestine did not vary significantly for the two formulations - pellets (3.1 h) and tablet (2.0 h). The pellets were found to have a longer residence time in the colon in all subjects as compared with the tablet, with mean colon transit time of 28 h for pellets and 15 h for the tablet. This study helped to highlight the differences in the *in vivo* behavior of multiparticulate and single unit dosage forms.

In another study, the GI transit of five small sized tablets in six patients with ulcerative colitis was monitored by Hardy et al (82), using radio-labeled imaging techniques. The mean gastric emptying time and small intestinal transit time were found to be 1.6 h and 3.4 h respectively. As the tablets were found to be retained more in the proximal colon for an appreciably long period of time of 6 h, it was concluded that small tablets were a good means of attaining site-specific delivery and controlled release in ulcerative colitis.

FUTURE PROSPECTS

Recent reports indicate interest in colon as a site where poorly absorbed drug molecules may have improved bioavailability. The distal colon is considered to have less hostile environment as well as enzyme activity compared to stomach and small intestine. The development of a dosage form that improves the oral absorption of peptide and protein drugs whose bioavailability is very low because of instability in the GI tract (due to pH or enzymatic degradation) is one of the greatest challenges for oral peptide delivery in the pharmaceutical field. Colon targeted multiparticulate systems like microspheres and nanoparticles can provide a platform for spatial delivery of candidates like peptides, proteins, oligonucleotides and vaccines.

However, drug release is not the end point of oral delivery. The bioavailability of protein drugs delivered at the colon site needs to addressed. The use of drug absorption enhancers into the drug delivery systems is likely to enhance therapeutic efficacy. Studies on drug absorption by the intestinal system have focused on drug transporters that mediate drug influx and efflux and agents which can enhance drug absorption. The colon segment is designed by nature mainly to expel metabolism products rather than to absorb nutrients. Therefore, more research that is focused on the specificity of drug uptake at the colon site is necessary. Such studies will be significant in advancing the cause of colon targeted delivery of therapeutics in future.

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