EMPRO

Current Research in Pharmaceutical Sciences

Available online at www.crpsonline.com

crps online CRPS

ISSN 2250 – 2688

Received: 12/05/2015
Revised: 29/05/2015
Accepted: 16/06/2015

Soni P, Chaudhary GP and Soni LK
School Of Pharmacy, Devi Aahilya Vishwavidhyalaya ,Indore (M.P.)

Enzyme Specific Drug Delivery System: A Potential Approach for Colon Targeting

Soni P, Chaudhary GP and Soni LK

ABSTRACT

Although oral delivery has become a widely accepted route of administration of therapeutic drugs, the gastrointestinal tract presents several formidable barriers to drug delivery. 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 but also for its potential for the delivery of proteins and peptides. To achieve successful colonic delivery, a drug needs to be protected from absorption or the environment of the upper gastrointestinal tract (git) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. Peptides, proteins, oligonucleotides and vaccines pose potential candidature for colon targeted drug delivery. The various strategies for targeting orally administered drugs to the colon include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, formulation of timed released systems, exploitation of carriers that are degraded specifically by colonic bacteria, bioadhesive systems and osmotic controlled drug delivery system. Certain plant polysaccharides such as amylose, inulin, pectin and guar gum remains unaffected in the presence of gastrointestinal enzymes and pave the way for the formulation of colon targeted drug delivery systems. Natural polysaccharides have been used as tools to deliver the drugs specifically to the colon. These polysaccharides remain intact in the physiological environment of stomach and small intestine but once the dosage form enters into colon, it is acted upon by polysaccharidases, which degrades the polysaccharide and releases the drug into the vicinity of bioenvironment of colon. However, they should be protected while gaining entry into stomach and small intestine due to enormous swelling and hydrophilic properties of polysaccharides. This has been achieved either by chemical crosslinking or by addition of a protective coat. The advantages of targeting drugs specifically to the diseased colon are reduced incidence of systemic side effects, lower dose of drug, supply of the drug to the biophase only when it is required and maintenance of the drug in its intact form as close as possible to the target site.

Keywords: Colon targeting, Colonic disease, Microbial triggered system, Approaches for colon delivery

1. INTRODUCTION

Oral administration of different dosage forms is the most commonly used method due to greater flexibility in design of dosage form and high patient acceptance, but the gastrointestinal tract presents several formidable barriers to drug delivery. Targeted drug delivery implies selective and effective localization of drug into the target at therapeutic concentrations with limited access to non target sites. A targeted drug delivery system is preferred in drugs having instability, low solubility and short half life, large volume of distribution, poor absorption, low specificity and low therapeutic index. Targeted drug delivery may provide maximum therapeutic activity by preventing degradation or inactivation of drug during transit to the target site. Meanwhile, it can also minimize adverse effects because of inappropriate disposition and minimize toxicity of potent drugs by reducing dose. An ideal targeted delivery system should be nontoxic, biocompatible and biodegradable and physicochemically stable *in vivo* and *in vitro*.[1] Targeted drug delivery to the colon is mainly for the treatment of colonic diseases, for drugs like proteins and peptides, for the treatment of diseases sensitive to circadian rhythms such as Asthma, Angina and Rheumatoid arthritis and for delivery of steroids, which absorbable in colon. The advent of slow release technologies increase the chances for a drug to be released in the colon and thus this organ has an important role to play in drug absorption from oral sustained release formulations.

Correspondence

Soni P
School Of Pharmacy, Devi Aahilya Vishwavidhyalaya ,Indore (M.P.)

E mail: sonipharma001@gmail.com

Colon-specific drug delivery system offers the following therapeutic advantages by (Girish et al[2], Chourasia et al[3], Etienne et al[4], Vyas and Roop.[5]

- Reducing the adverse effects in the treatment of colonic diseases (ulcerative colitis, colorectal cancer, crohn's disease etc.)
- By producing the 'friendlier' environment for peptides and proteins when compared to upper gastrointestinal tract.
- Minimizing extensive first pass metabolism of steroids.
- Preventing the gastric irritation produced by oral administration of NSAIDS.
- Delayed release of drugs to treat angina, asthma and rheumatoid arthritis [6,7]
- Formulations for colonic delivery are also suitable for delivery of drugs which are polar and/or susceptible to chemical and enzymatic degradation in the upper GIT, highly affected by hepatic metabolism, in particular, therapeutic proteins and peptides.

Colon specific drug delivery systems are also gaining importance for the delivery of protein and peptides due to several reasons as follow:

- Rapid development of biotechnology and genetic engineering resulting into the availability of protein and peptide drugs at reasonable cost.
- Proteins and peptide drugs are destroyed and inactivated in acidic environment of the stomach or by pancreatic enzymes in small intestine.
- Parental route is expensive and inconvenient.
- Longer residence time, less peptidase activity and natural absorptive characteristics make the colon as promising site for the delivery of protein and peptide drug for systemic absorption.
- Less diversity and intensity of digestive enzymes.
- Comparative proteolytic activity of colon mucosa is much less than that observed in the small intestine, thus colon targeting protects peptide drugs from hydrolysis, and enzymatic degradation in duodenum and jejunum, and eventually releases the drug into ileum or colon which leads to greater systemic bioavailability.

To achieve successful colon targeting it should overcome the following limitations (Jack et al[8-10])

- The location at the distal portion of the alimentary canal, the colon is difficult to access.
- Successful delivery requires the drug to be in solution before it arrives in the colon, but the fluid content in the colon is lower and more viscous than in upper git, which is the limiting factor for poorly soluble drugs.
- Lower surface area and relative tightness of the tight junctions in the colon can restrict drug transport across the mucosa in to the systemic circulation.

2. ANATOMICAL, PHYSIOLOGICAL & BIOCHEMICAL CHARACTERISTICS OF COLON

The large intestine extend from the ileocaecal junction to the anus which is divided into three main parts colon, rectum and anal canal. The colon constitute caecum, ascending colon , hepatic flexture, transverse colon, splenic flexture, descending colon and sigmoid colon (Fig 1). The average size of colon is 1.5 m long, the transverse colon is the longest and most mobile part and has an average diameter of about 6.5 cm. The wall of colon is consisting of four layers namely serosa, muscularis externa, submucosa and the mucosa. The serosa is the exterior coat of the large intestine. The muscularis externa is the major muscular coat of the large intestine which composed of an inner circular layer of fibers surrounding the bowel and of an outer longitudinal layer. The submucosa is the layer of connective tissue lies immediately beneath the mucosa . The mucosa has three parts epithelium, lamina propria and muscularis mucosa. Superior mesenteric artery supply blood to the proximal colon and the inferior mesenteric artery supplies blood to distal colon. [11,12]

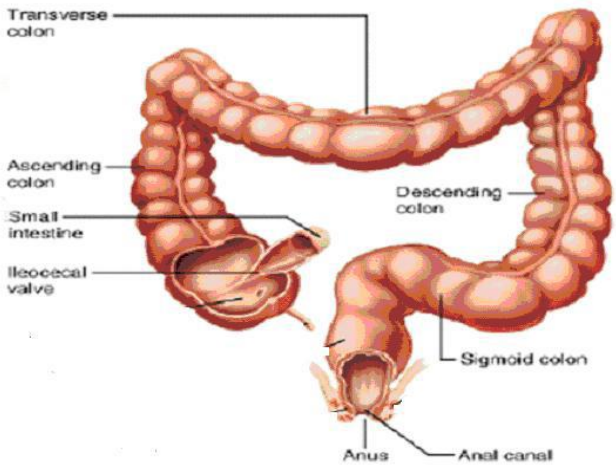

Figure1: Ascending, transverse, descending and sigmoid colon

2.1 Absorption of Drugs from Colon

The paracellular absorption is constant through the small intestine, but transcellular absorption appears to be limited to the small intestine, with negligible colonic absorption by this route. The epithelial cell junctions are very tight which may leads to poor paracellular absorption of many drugs in the colon. The drugs stay in contact with mucosa in colon for a longer period than in small

intestine which compensates the much lower surface areas of colon for absorption The colonic contents become more viscous with absorption of water as content travels through the colon. This cause a reduction in dissolution, and sluggish diffusion of dissolved drug through the mucosa .The colon is a more selective site for drug absorption as compare to small intestine because of the small extent of paracellular transport.

2.2 Factors Affecting Drug Absorption from Colon

The colon specific drug delivery primarily affected by two physiological factors, these are pH level and the transit time. The other factors which are also considered are as follows[13]

- Physical characteristic of drug (pka, degree of ionization)
- Colonic residence time as detected by gastrointestinal tract motility
- Degradation by bacterial enzymes and byproducts
- Selective and non selective bindings to the mucus
- Local physiological actions of drug
- Disease state
- Use of chemical absorption enhancers

3. COLONIC DISEASES

3.1 Inflammatory bowel disease (IBD)

Inflammatory bowel disease (IBD) is the communal terms for a group of idiopathic intestinal conditions include ulcerative colitis (UC) and Crohn'sdisease (CD). IBD is considered to be chronic relapsing disorder allied with uncontrolled inflammation within the gastrointestinal tract which may lead to the development of colorectal cancer later in life. CD and UC can be quite distinct, with different pathogenesis, inflammatory profiles, symptoms and treatment approach.[14,15]

3.1.1 Crohn's disease

Crohn's disease is a chronic inflammatory disease of the gastrointestinal tract; it is characterized by a granulomatous inflammation affecting any part of the tract, normally form fistulae.[16]

3.1.1.1 Pathology

Crohn's disease may occur anywhere in the gastrointestinal tract, although the most common pattern is an ileocolitis. The disease is often discontinuous, giving rise to so-called skip lesions. Isolated involvement of the mouth, oesophagus, stomach and anus is recognized but such cases are extremely rare.

3.1.1.2 Drugs used in treatment of CD

Azatioprine, Prednisolone, Budesonide, Metronidazole, Sulfasalazine, Infliximab, Mesalazine.

3.2 Ulcerative colitis

It is a chronic inflammatory disorder of colon limited to the large intestine as against the case with Crohn's Disease where any part of the alimentary tract may be involved. The condition usually manifests in the form of inflammation of the rectum extending further-up to colon. The inflammation may be limited to the left-hand side of the colon or extend to entire colon.[17]

3.2.1 Pathology

Macroscopic pathology of ulcerative colitis always involves the rectum but in about 40 percent of patients. Only 20 percent adults will have the whole colon involved, although this proportion rises to about 50 percent in children. In Microscopic pathology the inflammation of ulcerative colitis is largely confined to mucosa. The capillaries become dilated and congested and there is extravasation of red blood cell.

3.2.2 Drug used in treatment of this disease

Balsalazine sodium, Azathioprine, Olsalazine sodium, Budesonide, Mesalazine.[18]

4. STRATEGIES FOR TARGETING DRUGS TO COLON

The approaches for colon specific drug delivery system are prodrug or coated or matrix preparation.[19]

The commonly used approaches for colon specific drug delivery are:

1. pH dependent system
2. Time dependent system
3. Pressure dependent system
4. Microbial triggered system

4.1 pH dependent system

The pH-dependent system exploit the generally accepted view that pH of the human git increases progressively from the stomach (pH 1-2 which increases to 4 during digestion), small intestine (pH 6-7) and it increases to 7-8 in the distal ileum. The coating of pH-sensitive polymers to the tablets, capsules or pellets provide delayed release and protect the active drug from gastric fluid. The polymers used for colon targeting, how- ever, should be able to withstand the lower pH values of the stomach and of the

proximal part of the small intestine and also be able to disintegrate at the neutral or slightly alkaline pH of the terminal ileum and preferably at the ileocecal junction. These processes distribute the drug throughout the large intestine and improve the potential of colon targeted delivery systems.[20]

This can be achieved by means of coating of polymer that intact at lower pH of the stomach but that will be dissolved at neutral pH of the colon. The pH variation in the stomach and small intestine has previously been used to deliver drugs to small intestine by way of pH sensitive enteric coating. These polymer coats are recalcitrant to the acidic condition of the stomach but ionize and get dissolved above a certain threshold alkaline pH found in small intestine. Thus it is possible to apply same concept to deliver drugs to the terminal of ileum or colon by use of enteric polymers with a relatively high threshold pH for dissolution and subsequent drg release.[21]

4.1.1 Coating with pH-sensitive polymers

The majority of enteric and colon targeted delivery systems are based on the coating of tablets or pellets, which are filled into conventional hard gelatin capsules. However, during the early stage of drug development some new chemical entities (NCE's) present a challenge in testing for efficacy due to instability in gastric fluids because of irritation in the GIT. The limited amount of drug substance available during the early stage often precludes the development of a coated pellet or tablet formulation. Since the coating process is independent of the capsule contents, there are clear advantages resulting from the ability to coat a capsule. Thus, the oral pharmacological and or therapeutic efficacy of the NCE can be determined without resorting to extensive, time consuming and in many instances, impossible at this point in the development of the NCE. The GI residence time of the dosage forms is another important parameter for pH-dependent colon targeted drug delivery systems which is influenced by many physiological and other factors, [22,23] nevertheless, there are some generally accepted GI residence values for various parts of the GIT. [24] Most commonly used pH-dependent coating polymers are methacrylic acid copolymers, commonly known as Eudragit®, more specifically Eudragit® L and Eudragit® S . Eudragit® L100 and S 100 are copolymers of methacrylic acid and methyl methacrylate. The ratio of carboxyl to ester groups is approximately 1:1 in Eudragit® L100 and 1:2 in Eudragit® S 100. The polymers form salts and dissolve above pH 5.5 and disperse in water to form latex and thus avoid the use of organic solvents in the coating process. The water solubility of the Eudragit® S depends on the ratio of free carboxyl groups to the esterifies groups. The critical factor that influences the performance of these polymers is the pH value at which dissolution occurs. Polymers with ionizable phthalic acid groups dissolve much faster and at a lower pH than those with acrylic or methacrylic acid groups. The presence of plasticizer[24] and the nature of the salt[25,26] in the dissolution medium also influence the dissolution rate of Eudragit®. In addition, the permeability of the film formed may depend on the type of solvent used to dissolve Eudragit®[27]. Colon targeted drug delivery systems based on methacrylic resins has described for insulin[28], prednisolone[29], quinolones[30], salsalazine[31-34], cyclosporine[35], beclomethasone dipropionate[36] and naproxane[37]. Khan et al.[38] prepared lactose-based placebo tablets and coated using various combinations of two methacrylic acid polymers, Eudragit® L100-55 and Eudragit® S100 by spraying from aqueous systems. The Eudragit® L100-55 and Eudragit® S100 combinations studied were 1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 1:5 and 0:1. The coated tablets were tested *in vitro* for their suitability for pH dependent colon targeted oral drug delivery. The same coating formulations were then applied on tablets containing mesalazine as a model drug and evaluated for *in vitro* dissolution rates under various conditions. The disintegration data obtained for the placebo tablets demonstrate that disintegration rate of the studied tablets is depends on the polymer combinations used to coat the tablets, pH of the disintegration media and the coating level of the tablets. Dissolution studies performed on the mesalazine tablets further confirmed that the release profiles of the drug could be manipulated by changing the Eudragit® L100-55 and Eudragit® S100 ratios within the pH range of 5.5 to 7.0 in which the individual polymers are soluble respectively, and a coating formulation consisting of a combination of the two copolymers can overcome the issue of high GI pH variability among individuals. The results also demonstrated that a combination of Eudragit® L100-55 and Eudragit® S100 could be successfully used from aqueous system to coat tablets for colon targeted drug delivery of drugs and the formulation can be adjusted to deliver drug at any other desirable site of the intestinal region of the GIT on the basis of pH variability. Lorenzo-Lamosa et al.[39] prepared and demonstrated the efficacy of a system, which combines specific biodegradability and pH dependent release behavior. The system consists of chitosan microcores entrapped within acrylic microspheres containing diclofenac sodium as model drug. The drug was efficiently entrapped within the chitosan microcores using spray drying and then microencapsulated into Eudragit® L-100 and Eudragit® S-100 using an oil-in-oil solvent evaporation method. Release of the drug from chitosan multireservoir system was adjusted by changing the chitosan molecular weight or the type of chitosan salt. Furthermore, by coating the chitosan microcores with Eudragit®, perfect pH-dependent release profiles were attained. Numerous Eudragit® coated oral dosage forms of salsalazine are currently in use for the treatment of ulcerative colitis and Chron's disease. [40-46] Two enteric polymers Eudragit® L-30 D-55 and Eudragit® FS 30 D were studied, which were designed to achieve enteric properties and colonic release respectively. Dissolution studies demonstrated that capsules coated

with Eudragit® L-30 D-55 were gastro resistant for 3 h at pH 1.2 and capsules coated with Eudragit® FS 30 D were resistant for a further 1 h at pH 6.8. Several methacrylated derivatives of Eudragit® S with different degrees of substitution were prepared for evaluation as potential coatings for colon targeted drug delivery. Water vapour transmission, *in vitro* dissolution and stability in function of pH were investigated using the technique of isolated films. The data presented demonstrate relative differences in physicochemical characteristics due to differences in degree of substitution.[47] Ondansetorn and Budesonide drugs, which are used for local treatment of intestinal disorders, were efficiently entrapped in a new microparticulate system, which combines pH-dependent and controlled drug release properties. This system was formulated by drug loaded cellulose acetate butyrate (CAB) microspheres coated by an enteric polymer (Eudragit® S). Both, CAB cores and pH-sensitive microcapsules were prepared by the emulsion-solvent evaporation technique. The *in vitro* drug release studies of pH-sensitive microcapsules containing the hydrophobic cores showed that no drug was released below pH 7. After that, CAB microspheres efficiently controlled the release of Budesonide, the release behavior being affected by the different polymer concentration used in their preparation[48]. The disadvantage of this approach is the lack of consistency in the dissolution of the polymer at the desired site. Depending on the intensity of the GI motility, the dissolution of the polymer can be complete deep in the colon or at the end of the ileum. Moreover, many factors such as the presence of short chain fatty acids, residues of bile acids, carbon dioxide or other fermentation products can reduce the colonic pH to approximately 6 and call its pH as a trigger into question. Ashford et al.[49] have shown that pH-sensitive polymers are not suitable for colon targeted drug delivery systems due to poor site specificity. The long lag times at the ileocaecal junction and fast transit indicate that a single unit may not be the best dosage form for a colon targeted drug delivery system. The late disintegration of a single-unit dosage form creates a particular problem due to the abnormal release of contents and will results in loss of much of the opportunity for local action or absorption in the proximal colon. This problem can be partially rectified by targeting multiple-unit dosage forms. The microparticulate system consisted of Budesonide-containing hydrophobic cores, microencapsulated within an enteric polymer was prepared by Rodriguez et al.[50] which solubilizes at above pH 7, thus combining pH-sensitive and controlled-release properties. Colonic injury and inflammation were assessed by measuring colon/body weight ratio, myeloperoxidase activity, and by scoring macroscopic and histological damage in colitic rats. Rats were treated orally with Budesonide, included in the developed system, once a day for 4 days after the induction of inflammation. A Budesonide suspension and Budesonide-containing enteric microparticles were included as control formulations in the experimental design. The administration of the new Budesonide delivery system significantly reduced the colon/body weight ratio compared with the administration of control formulations. Similarly, myeloperoxidase activity and macroscopic and histological damage of the inflamed colonic segments decreased significantly when the Budesonide formulation was administered compared with the results obtained after oral administration of the drug suspension. There were no significant differences, however, when the new treatment was compared with the control formulation consisting of simple enteric microparticles. Markus et al [51] developed a multi-unit dosage form containing 5-ASA for the treatment of ulcerative colitis. Pellets were prepared by a granulation and spheronization process and then coated with a new pH sensitive poly(meth)acrylate copolymer (Eudragit® FS 30D) to achieve site specific drug release close to the ileocaecal valve. From the dissolution studies it was concluded that pellets released rapidly at pH values above 7.5. Between 6.8 and 7.2 drug release was found to be zero order, while at pH 6.5 and below no release occurred. In a biorelevant medium, which simulates the fasting proximal small intestine fluid, it was shown that neither surfactants (sodium taurocholate and lecithin) nor changes in ionic strength trigger drug release. Compared to 5-ASA pellets coated with the well established Eudragit® S, and to currently marketed products licensed for the treatment of ulcerative colitis, the multi-unit dosage form coated with the new polymer exhibited an *in vitro* dissolution profile more appropriate to the pH profile of the ileum and the colon observed in ulcerative colitis patients.

EUDRACOL is a novel pH and time controlled multiple unit colon drug delivery systems in which the pellets coated with Eudragit RL /RS and Eudragit FS 30D. Caffeine is used as marker drug for pharmacokinetic studies using the multiparticle principle and delayed release in the colon; reduction of dosing frequency may be achieved. Due to its specific coating structure, the Eudracol system offers a new dimension for colon drug targeting via the oral route.[52] 5-ASA pellets were coated with the enteric coating solution containing different ratios at Eudragit L-100 and Eudragit S-100 for colon drug delivery. The release of 5-ASA is depending on the thickness of the layer and the ratio of Eudragit copolymers[53]. PH-sensitive hydrogels were prepared for colonic delivery of therapeutic peptides and proteins. New pH-sensitive glycopolymers were developed by free radical polymerization of methacrylic acid and 6-hexandiol diacrylate and 6- hexandiol propoxylate diacrylate.[54]

4.1.2 Embedding in pH-sensitive matrices

Extrusion-spheronization and pelletization have been used for the preparation of pH-sensitive matrix pellets for colon targeted drug delivery[55].Chaurasia et al.[3] studied the effects of three independent variables (amounts of Eudragit® S, citric acid and spheronizing time) on pellet size, shape (roundness and aspect ratio), and drug release was studied with central composite design.

Nykanen et al.[56] used ibuprofen as model drug and Eudragit® S and Aqoat AS-HF as enteric polymers for developing site-specific systems for release of a drug in the lower part of the small intestine or in the colon. The target of this study was to investigate whether drug release rate from enteric matrix granules could be influenced by using organic acids as excipients. It was concluded that although inclusion of an organic acid in a formulation retarded *in vitro* release of the model drug, no corresponding effect was evident in case of *in vivo* studies.

4.2 Time dependent system

The average transit time in the stomach is 2 hr which may vary, while in the small intestine it is relatively constant around 3hr. The typical transit time varies from 20 to 30h.Time dependent drug delivery system allow the drug release after a set time delay. For the colon targeted drug release the lag time should similar to the time taken for the system to reach the colon. The lag time of 5 hr is usually considered sufficient on the basis of relatively constant transit time in the small intestine (3hr); Pulsicap was the first formulation developed based on this approach. Time dependent approach was also been used for chronopharmacotherapy using nifedipine and coating with polyethylene oxide- polyethylene glycol mixtures which release the drug in colon.[57,58] Eudragit L100 along with channeling agent like sodium chloride has been effectively confirmed for achieving colon target drug delivery based on this approach. Hydroxy propyl methyl cellulose has been used for colon specific drug delivery of pseudoephedrine HCL using this approach. Hydroxy propyl ethyl cellulose, Hydroxy propyl methyl cellulose acetate succinate were also been used for time dependent colon specific drug delivery.[59,60] HPMC along with pectin has also been shown to produce promising result for colon drug delivery system for sennosides which is used as an herbal purgative. The hydrogel based capsule was reported which swells after definite time and allow drug release after lag time successfully in colon, hence by modifying hydrogel composition and size, lag time could be varied.[61]

Colon drug delivery system of diclofencac sodium (DS) was developed using time dependent approach. In this, diclofencac sodium tables were coated with ethylcelluese in ethanol solution cooling diethyl phthalate as a plasticizer and PEG 400 as channeling agent. The lag time of DS release wa sprimarily controlled by thickness of ethycellulose coating layer. By increasing the thickness of the coating layer, longer the lag time of DS release.[53]

Formulation of fast release enteric coated tablets for colon drug delivery using two different approaches .The first one is using super disintegrant and the second one is based on osmogen. In the first approach core tablets (celicoxib as a model drug) were prepared using different concentrations of super disintegrant like cross-linked PVP. In second approach concentrations tablets were prepared using potassium chloride and sodium chloride as osmogen. Then they are coated with Eudragit L-100: Eudragit S-100 in the ratio of 1:5 to achieve a desired thickness. The tablets with super disintegrant are fast released where the tablets with osmogen are sustain released. The coat weight determines the lag phase that required eliminating the release in stomach and small intestine[62]. Hydroxy Propyl Methyl Cellulose (HPMC) compression coated tablets of 5-fluorouracil were studied for colon drug delivery that based on time-dependent approach. In this, the core tablet was prepared by wet granulation method and then coated with 50% of HPMC/lactose coat powder by compression-coating method. Drug release characteristics were evaluated in distilled water by using a Chinese pharmacopoeia rotatable basket method.[63] Gazzaniga et al [64] described a novel oral time based drug release system for colon-specific delivery. The system designed to exploit the relatively constant small intestinal transit time of dosage forms consists of drug-containing cores coated with three polymeric layers. The outer layer dissolves at pH > 5, then the intermediate swellable layer, made of an enteric material. The system provides the expected delayed release pattern, as also indicated by the preliminary in vivo studies on rats. Several other drug delivery systems have developed that rely upon the relatively constant transit time of small intestine.[65-68] A novel delivery system was developed for delivering drugs to the colon by selecting polymethacrylates with appropriate pH dissolution characteristics for the distal end of the small intestine. Pellets were prepared by powder layering of 5-ASA on nonpareils (0.5-0.6 mm) in a conventional coating pan. A delivery system called the Time Clock® has been exploited to release the drug in the colon [69-72]. 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. In a study with human volunteers, it was shown that the lag time was independent of gastric residence time and hydrophobic film redispersing did not appear to be influenced by the presence of intestinal digestive enzymes or by mechanical action of the stomach. A capsule consisting of EC was prepared and evaluated for site-specific drug delivery to the colon.[73] 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 polymer forces the EC cap to disintegrate, thereby releasing the drug.

4.3 Pressure dependent system

Peristaltic movements of intestines along with gastric contractile activity are responsible for the propulsion of intestinal contents. These peristaltic movements constitute elevated luminal pressure conditions in the colon. The design of pressure controlled

drug delivery system is based upon peristaltic movements. Intensity and duration of this pressure varies with the muscular contractions in the visceral organs.[74] It consists of a capsule shaped suppositories coated with the water insoluble polymer like ethyl cellulose (EC). Once taken orally, they behave like balloon of ethyl cellulose because the base of the capsule liquefy at the body temperature.[75,76] The thickness of the ethyl cellulose membrane play a very vital role in the disintegration of the capsule. The size and density of the capsule may also affects the system. The preferred thickness of the capsule wall is about 35-60 μm. The viscosity of the luminal content is higher in the colon than the small intestine because of re-absorption of water from the colon, so that drug dissolution in the colon could present a problem for colon-specific oral drug delivery system. When the pressure controlled capsule was administered to human volunteer, the lag time of three to five hours in relation of drug absorption were noted. And, found that disintegration of capsule achieved as the luminal pressure hikes. Pressure controlled colon delivery capsule (PCDC) containing 5-ASA was prepared and was administered orally to beagle dogs. After administration, 5-ASA appeared into the systemic circulation at 3-5 h that corresponds to the colon arrival time confirmed with sulfasalazine.[77] The relationship between *in vitro* drug release characteristics from colon delivery systems and *in vivo* drug absorption was investigated using three kinds of delayed-release systems. 5-ASA, tegafur and carbamazepine were selected as model drugs. Pressure-controlled colon delivery capsules for liquid preparations, time-controlled colon delivery capsules for liquid and solid preparations and Eudragit® S coated tablets for solid preparations were used in this study. At first, *in vitro* dissolution tests for all preparations were performed. Drug release from solid preparations was delayed compared to that from liquid preparations with all three drugs. From the findings of the study it was concluded that drug release from colon delivery systems and drug dissolution in the colonic lumen are very important factors for the systemic availability of drugs from the colon delivery systems.[78,79]

4.4 Microbial triggered system

These systems are based on the exploitation of the specific enzymatic activity of the microflora (enterobacteria) present in the colon. The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substrates such as carbohydrates and proteins that escape the digestion in the upper GIT.[80] Bacterial count in colon is much higher around 10^{11}-10^{12} CFU/mL with some 400 different species which are fundamentally aerobic, predominant species such as *Bacteroides, Bifidobacterium and Eubacterum* etc., whose major metabolic process occurring in colon are hydrolysis and reduction. The enzymes present in the colon are:

- Reducing enzymes: Nitroreductase, Azoreductase, N-oxide reductase, sulfoxide reductase, Hydrogenase etc.,
- Hydrolytic enzymes: Esterases, Amidases, Glycosidases, Glucuronidase, sulfatase etc.

The vast microflora fulfills its energy needs by ferment in various types of substrates that have been left undigested in the small intestine, e.g. di- and tri-saccharides, polysaccharides etc. For this fermentation, the microflora produces a vast number of enzymes like glucoronidase, xylosidase, arabinosidase, galactosidase, nitroreductase, azareducatase, deaminase, and urea dehydroxylase. Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be a more site-specific approach as compared to other approaches. These polymers shield the drug from the environments of stomach and small intestine, and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by microorganism, or degradation by enzyme or break down of the polymer back bone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer .[81,82] Natural materials, fundamentally those that are polysaccharide-based, offer a workable alternative to safety problem, material includes chitosan, amylose, dextran, guar gum and pectin.[83,84] Biodegradable polymers degrade in vivo, either in presence of enzyme or non enzymatically, to produce products which are non toxic and biocompatible. The microflora composition remains relatively constant across a diverse human population. The colon targeting is achieved by this approach in different ways:

4.4.1 Covalent linkage of the drug with a carrier

It involves the formation of a covalent linkage between drug and carrier in such a manner that upon oral administration the moiety remains intact in the stomach and small intestine. This approach chiefly involves the formation of prodrug, which is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation in the biological environment to release the active drug. Formation of prodrugs has improved delivery properties over the parent drug molecule. The problem of stability of certain drugs from the adverse environment of the upper GIT can be eliminated by prodrug formation, which is converted into parent drug molecule once it reaches into the colon. Site specific drug delivery through site specific prodrug activation may be accomplished by the utilization of some specific property at the target site, such as altered pH or high activity of certain enzymes relative to the non-target tissues for the prodrug-drug conversion.

4.4.2 Azo bond conjugates

The intestinal microflora is characterized by a complex and relatively stable community of microorganism, many with

physiological functions, which play vital roles in health and disease. In addition to protection of the patient against colonization of the intestinal tract by potentially pathogenic bacteria, the indigenous microflora are responsible for a wide variety of metabolic processes, including the reduction of nitro and azo groups in environmental and therapeutic compounds.[85,86] Sulphasalazine was introduced for the treatment of rheumatoid arthritis and anti-inflammatory disease. Chemically it is salicylazosulphapyridine (SASP), where sulfapyridine is linked to a salicylate radical by an azo bond.[87] When taken orally, only a small proportion of the ingested dose is absorbed from the small intestine and the bulk of the sulphasalazine reaches the colon intact. There it is split at the azo bond by the colonic bacteria with the liberation of sulphapyridine (SP) and 5-ASA. However sulphapyridine is seems to be responsible for most of the side effects of sulphasalazine and hence various new approaches for the treatment of IBD have emerged.

The need for less toxic carrier moieties has led to the development and testing of a number of other azo-bond prodrugs. By replacing the carrier molecule with others, a number of prodrugs of 5-ASA can be prepared e.g. p-aminohippurate (4-amino benzoyl glycine) in ipsalazine, 4-amino benzoyl-b-alanine in balsalazine,[88] p-aminobenzoate in HB-313[89] or a nonabsorbable sulphanilamide ethylene polymer in poly-ASA.[90] The most interesting prodrug is olsalazine (OSZ) which is a dimer representing two molecules of 5-ASA that are linked via an azo bond. When olsalazine reaches the large intestine, it is cleaved releasing two molecules of 5-ASA for every mole of olsalazine administered. This prodrug is absorbed intact from the human GIT to only a very limited extent and, as with SASP, 5-ASA and acetyl-5-ASA are recovered in the feces following oral administration of OSZ.[91,92] It has been shown clinically that an intact GIT and a normal microflora population are required for effective splitting of OSZ.[93,94] Fecal recovery of 5-ASA has been found to be virtually identical to an equamolar dose of SASP.[95] Clinical trials have been encouraging although watery diarrhea has emerged as new and troublesome side effect, which generally affects about 15% of the patients. This side effect appears to be related to a combination of gastrointestinal transit and a stimulation of small intestinal secretion.[96,97] A second azo bond prodrug developed is balsalazine, which is 5-ASA azo-linked to 4-aminobenzoyl- β -alanine. This carrier is designed to be inherently less toxic than SP while maintaining the poor absorbability of the prodrug from the upper GIT. The promoiety is only minimally absorbed following azo-reduction in the colon. Clinical trials suggest that balsalazine is useful in maintaining remission in the ulcerative colitis [98] with fewer side effects than are associated with SASP maintenance therapy. Another prodrug called ipsalazine has also been synthesized and tested as a carrier for 5-ASA. Despite promising pharmacokinetic data, ipsalazine has not been developed further.[99]

Biorecognizable HPMA copolymer conjugates for colon-specific delivery of 9-aminocamptothecin (9-AC) was designed. They hold 9-AC bound via spacers containing amino acid residues and aromatic azo bonds. In vitro release profiles of 9-AC from HPMA copolymer conjugates were evaluated under artificial conditions that simulated large intestinal azoreductase and peptidase activities. The studies indicated that the azo bond was reduced first, followed by the release of unmodified 9-AC from the 9-AC containing fragment by peptidases. Release profiles depended on the chemical structure of the peptide part of the spacer. Conjugates containing leucylalanine showed high colon-specific release of 9-AC when compared to alanine containing conjugates .[100]

4.4.3 Glycoside conjugates

Steroid glycosides and the unique glycosidase activity of the colonic microflora form the basis of a new colon targeted drug delivery system. Drug glycosides are hydrophilic and thus, poorly absorbed from the small intestine. Once such a glycoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to be absorbed by the colonic mucosa. The major glycosidases identified in human feces are β-D-galactosidase, β -D-glucosidase, α-L-arabinofuranosidase, β-D-xylopyranosidase.[101] These enzymes are located at the brush border and hence access to the substrate is relatively easy. In the plant kingdom numerous compounds are found as glycosides. Certain drugs act as glycon and can be conjugated to different sugar moieties which results in the formation of glycosides. Due to the bulky and hydrophilic nature of these glycosides, they do not penetrate the biological membrane upon ingestion.[102] Various naturally occurring glycosides, e.g. the sennosides, have been used for laxative action for ages. When taken orally, intact sennosides are more efficient as laxative than sugar free aglycones. These sennosides are activated by colonic microflora to generate rhein anthones, which gives the desired laxative effect.[103] Glycosidase activity of the GIT is derived from anaerobic microflora in the large bowel or the sloughed or exfoliated cells of the small intestine.[104,105] Friend and Chang[106] prepared dexamethasone-21-β-glucoside and prednisolone-21- β -glucoside for delivery of these steroids to the colon.

Hydrolysis of prodrugs by β-glucosidase and fecal homogenates *in vitro* released the free steroids. Glucosides were administered to rats intragastrically to determine when and where the free steroids were released. Unmodified dexamethasone and prednisolone were also given to rats intragastrically to compare absorption of the glucosides with the free steroids. Both glucosides were found to reach the rat lower intestine in 4-5 h, where they were rapidly hydrolyzed, releasing the free steroids. *In vivo* studies on dexamethasone- β -D-glucoside revealed that nearly 60% of an oral dose of glucoside reached the caecum whereas in case of prednisolone- β -D-glucoside, only 15% reached to the caecum. When free steroids were administered orally, they were almost absorbed in the small intestine and less than 1% of oral dose reached at the colon. The influence of prodrug structure on specificity of glycoside/glycosidase based colon-specific drug delivery was studied by preparing nine steroid glycosides, measuring their relative lipophilicities and hydrolyzing them with bacterial glycosidases from rat intestines.[107] The 21-yl β-D-glucosides and galactosides of dexamethasone, prednisolone, hydrocortisone and fludrocortisone and 21-yl β-D-cellobioside of prednisolone were prepared by a modified Koenigs-Knorr reaction. The deacetylated glycoside prodrugs along with the p-nitrophenyl derivatives of β-D-glucoside, galactoside and cellobioside were

subjected to hydrolysis by the contents of the rat stomach, proximal small intestine (PSI), distal small intestine (DSI) and caecum. All the prodrugs were hydrolyzed slowly by PSI and stomach contents, more rapidly by contents of the DSI, and most rapidly by caecal contents. *In vitro* studies performed specifically on dexamethason-β-D-glucoside[108-110] revealed that both GIT tissues and GIT contents of guinea pig showed β-glucosidase activity. Among the tissues maximum activity was seen in tissues of PSI whereas among the contents maximum activity was seen in the caecum and the colon. For *in vivo* studies experimental IBD was induced using degraded carrageenan in guinea pig. 0.65 mol/kg dexamethason- β -D-glucoside was equally effective as 1.3 mol/kg of dexamethason alone in reducing the total number of ulcers. The results indicated that a lower dose of dexamethasone, administered, as its glucoside prodrug could be equally efficacious relative to higher dose of dexamethasone.

4.4.4 Glucuronide conjugates

Glucuronide and sulphate conjugation is the major mechanisms for the inactivation and preparation for clearance of a variety of drugs. Bacteria of the lower GIT, however, secrete β-glucuronidase and can deglucuronidate a variety of drugs in the intestine.[111] Since the deglucuronidation process results in the release of active drug and enables its reabsorption, glucuronide prodrugs would be expected to be superior for colon targeted drug delivery.

Morphine-dependent rats were used to evaluate the effects of the narcotic antagonists, naloxone and nalmefene, and their glucuronide conjugates on the gastrointestinal tract and various parameters of brain-mediated withdrawal. When administered subcutaneously nalmefene hydrochloride caused a dose-dependent tail skin temperature increase, whereas nalmefene glucuronide was ineffective. Nalmefene precipitated brain-mediated morphine withdrawal at doses as low as 10 mg/kg, whereas nalmefene glucuronide was ineffective at doses as high as 1 mg/kg. After per oral administration of the drugs, naloxone hydrochloride and nalmefene hydrochloride caused diarrhea, withdrawal behavior and tail skin temperature responses by 15 minutes. In contrast, after per oral administration of the glucuronide conjugate of either narcotic antagonist, diarrhea was delayed for 75 to 203 minutes. This latency probably reflects the required transit time to the lower gastrointestinal tract. About 0.2 to 0.5% of the dose of the narcotic antagonist administered orally as the glucuronide was absorbed systemically. These results indicate that per oral administration of the glucuronide conjugates of naloxone and nalmefene results in delivery of the narcotic antagonists to the colon.[112] Haeberlin et al [113] prepared a dexamethasone- β -D-glucuronide prodrug.

The treatment of ulcerative colitis was improved by the synthesis of budenoside and dexamethasone conjugates of glucuronic acid and dextran[114,115] The system showed excellent performance in ulcerative colitis and reduces the systemic toxicity of corticosteroids including adrenal suppression by immurement of activity of the drug in large intestine. The preclinical efficacy of dexamethasone- β -D-glucuronide was confirmed in the rat model of ulcerative colitis and Chron's colitis.[116] Nolen et al[117] investigated the steady-state pharmacokinetics of corticosteroid delivery from glucuronide prodrugs in normal and colitic rats. Two prodrugs, dexamethasone- β-D-glucuronide (DXglrd) and Budesonide- β -D-glucuronide (BUDglrd) were administered by intragastric infusion to conventional and colitic rats. In addition, dexamethasone and Budesonide were administered either intragastrically or subcutaneously to healthy and colitic rats and colon-specific delivery was assessed using the drug delivery index. In conventional rats, drug delivery indices for DXglrd ranged from about five to as high as 11 in the luminal contents relative to dexamethasone administered subcutaneously or intragastrically. Drug delivery index values were also elevated in the mucosa of both healthy and colitic rats following intragastric administration of DXglrd. Budesonide was delivered somewhat less effectively from BUDglrd to the rat large intestine than was dexamethasone from DXglrd.

4.4.5 Cyclodextrin conjugates

Cyclodextrins (CyDs) are cyclic oligosaccharides consisted of six to eight glucose units through α -1,4 glucosidic bonds and have been utilized to improve certain properties of drugs such as solubility, stability and bioavailability. The interior of these molecules is relatively lipophilic and the exterior relatively hydrophilic, they tend to form inclusion complexes with various drug molecules[118-122] They are known to be barely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however, they are fermented by colonic microflora into small saccharides and thus absorbed in the large intestine.[123-125] Because of their bioadaptability and multi-functional characteristics, CyDs are capable of alleviating the undesirable properties of drug molecules in various routes of administration through the formation of inclusion complexes. In an oral drug delivery system, the hydrophilic and ionizable CyDs can serve as potent drug carriers in the immediate release and delayed release-formulations, respectively, while hydrophobic CyDs can retard the release rate of watersoluble drugs. It has been proved through a study in healthy human volunteers that β-CyDs are meagrely digested in small intestine but are completely degraded by the microflora of the colon. Most bacterial strains that are isolated from human being are capable of degrading CyDs. It has been proved by their ability to grow on cyclodextrins by utilizing them as the sole carbon source and by the stimulation of cyclodextrinase activity by as low as 2-4 h of exposure to cyclodextrins. This property of the drug may be exploited for the formation of colon targeted drug delivery systems. An anti-inflammatory drug biphenylacetic acid (BPAA) as model drug was selectively conjugated onto one of the primary hydroxyl groups of α -, β - and γ- CyDs through an ester or amide linkage, and the *in vivo* drug release behavior of these prodrugs in rat gastrointestinal tract after oral administration was investigated. The CyD prodrugs were stable in rat stomach and small intestine and negligibly absorbed from these tracts. Three to six h after oral administration, most of the prodrugs had moved to the caecum and colon. The α-

and γ-CyD amide prodrugs were hydrolyzed to the maltose conjugate in the caecum and colon, and these prodrugs and the conjugates were negligibly absorbed. On the other hand, the α-and γ-CyD ester prodrugs produced BPAA in the caecum and colon, and BPAA appeared in the blood after 3-6 h. Both β -CyD amide and ester prodrugs released only small or negligible amounts of the maltose conjugate or BPAA in the caecum and colon within 24 h, probably due to the low solubility in the biological media. Further, the anti-inflammatory effect of the γ-CyD ester prodrug was evaluated using the model of carageenan-induced acute edema in rat paw and compared with those of BPAA alone and the BPAA/ β -CyD complex prepared by the kneading method in a molar ratio of 1:1. In the case of β -CyD complex, a rapid anti-inflammatory response was observed from the small intestine after a fast dissolution of the complex. In sharp contrast, the γ-CyD ester prodrug required a fairly long lag time to exhibit the drug activity, because BPAA was produced after the prodrug had reached the caecum and colon. These results clearly suggest that the CyD prodrug approach could provide a versatile means for constructions of not only colon-specific delivery systems but also delayed-release system of certain drugs.[126-127] Hiramaya et al [128] prepared two CyD conjugates where one primary hydroxyl group of β -CyDs was substituted by BPAA through an ester or amide linkage. Aqueous solubility of the conjugates was lower than those of other drug or parent compound. The amide conjugate was stable in aqueous solution and in rat biological fluids and gastrointestinal contents. The ester conjugate released the drug preferentially when incubated with the contents of caecum or colon, whereas no appreciable drug release was observed on incubation with the contents of either stomach or intestine in intestinal or liver homogenates or in rat blood. Prednisolone, a typical glucocorticoid, has been widely used for the treatment of IBD. However, when Prednisolone is administered orally, a large amount of the drug is absorbed from the upper GIT and causes systemic side effects. The anti-inflammatory effect and systemic side effect of the prednisolone succinate/alpha-cyclodextrin ester conjugate after oral administration were studied using IBD model rats. The systemic side effect of the conjugate was much lower than that of prednisolone alone when administered orally. The lower side effect of the conjugate was attributable to passage of the conjugate through the stomach and small intestine without significant degradation or absorption, followed by the degradation of the conjugate site-specifically in the large intestine.[129] Two of the parent CyDs, α-CyD and β -CyDs are known to be parenterally unsafe due to severe nephrotoxicity[130,131]. Hydrophobic CyD may be useful in various controlled release formulations of water-soluble drugs including peptides and protein drugs.[132]

4.4.6 Dextran conjugates

Dextran ester prodrug was prepared and *in vitro* release revealed that release of naproxan from prodrug was several folds higher in caecum homogenates than in control medium or homogenates of the small intestine of pig.[133,134] The bioavailability of naproxan after oral administration of a dextran T-70-naproxan ester prodrug in pigs was assessed by Harboe et al[135] Compared to the administration of an oral solution of an equivalent dose of naproxan the average absorption fraction for the conjugate amounted to 91%. It was established that several features of the prodrug indicated that naproxan was released from the prodrug prior to systemic absorption and that drug activation involved the action of one or more enzyme systems located in the gastrointestinal tract. It was observed in rabbits, the plasma concentration-time curves for the conjugate were characterized by an initial lag time of about 2-3 h, whereas naproxan was detected in plasma immediately after per oral administration of the drug compound per se. The distribution of the prodrug along the GIT at various times after conjugate administration was assessed qualitatively by HPLC analysis of conjugated and free naproxan in various segments of the GIT. From these experiments it was suggested that drug regeneration was effective in the bowel below the ileum.

Dextran ester prodrugs of metronidazole have been prepared and characterized.[136-138] Mcleod et al[139] synthesized dextran ester prodrugs of dexamethasone and methylprednisolone and proved the efficacy of the prodrugs for delivering drugs to the colon. In this study, methyl prednisolone and dexamethasone were covalently attached to dextran by the use of a succinate linker. In addition, dexamethasone was attached by glutaric acid to investigate the effect of linker molecule on hydrolysis kinetics. The kinetics of degradation of the hemiester and corresponding dextran conjugates were measured as a function of pH and temperature. Intermolecular migration of the linker molecule from the 21- to the 17-position on the glucocorticoid occurred in all three hemiester, although to a greater extent in methylprednisolone-hemiester. The dextran conjugates were also incubated at 37°C; pH 6.8 and the chemical degradation half-lives were determined. Dexamthasone-21-hemisuccinate showed half life of 75 h, dexamethasone-glutaratedextran exhibited half life of 103 h, while methylprednisolone-succinate-dextran showed half life of 82 h. Glucocorticoids remain the foundation of therapy for acute ulcerative colitis despite systemic side effects that limit their use. Prodrugs that selectively deliver glucocorticoids to the colon may lower the required dose and side effects.

4.4.7 Amino-acid conjugates

Due to the hydrophilic nature of polar groups like -NH_2 and -COOH, that is present in the proteins and their basic units (i.e. the amino acids), they reduce the membrane permeability of amino acids and proteins. Various prodrugs have been prepared by the conjugation of drug molecules to these polar amino acids.[140-143] Non-essential amino acids such as tyrosine, glycine, methionine and glutamic acid were conjugated to Salicylic acid (SA). The salicyluric acid (the glycine conjugate of SA) was found to be metabolized to SA by the microorganisms of the intestinal flora of rabbit and dog. The prodrug was absorbed into the systemic circulation from the upper GIT and hence it was proved unsuitable for delivery of drugs to the colon. By increasing the hydrophilicity and chain length of the carrier amino acid and decreasing the membrane permeability of conjugate, Nakamura et al[140] prepared salicylic glutamic acid conjugates . This conjugate showed splendid results with minimal absorption and degradation in the upper GIT and proved suitable for colon targeted delivery of SA.

Polymeric prodrugs have developed using a spacer coupling 5-ASA via 5-amino function by an azo bond. The spacer 5-ASA conjugates is then covalently linked to poly (methyl vinyl ether/comaleic anhydride) and poly (1-vinyl-2-pyrrolidone co-maleic anhydride) and also to chloroformate-activate derivatives of dextran and poly [(2-hydroxyethyl) aspartamine]. The release of 5-ASA from polymeric prodrugs was depended upon the structure of the polymeric backbone.[144] Despite the fact that all these polymeric prodrugs can deliver 5-ASA successfully to the large intestine, 5-ASA may not be the drug of choice for these systems. Indeed, the required dose of 5-ASA ranges from 0.5 to 3g daily, and since the drug makes less than 10% of the total weight of the prodrug; a very large amount would need to be taken orally. Poly-(L-aspartic acid) has been investigated as carrier for colon targeted delivery of dexamethasone.[145,146]

4.4.8 Coating with biodegradable polymers

The bioenvironment inside the human GIT is characterized by the presence of complex microflora especially the colon that is rich in microorganisms that are involved in the process of reduction of dietary component or other materials. Drugs that are coated with the polymers, which are showing degradability due to the influence of colonic microorganisms, can be exploited in designing drugs for colon targeting. These bacterial degradable polymers especially azo polymers have been explored in order to release an orally administered drug in the colon. Actually, upon passage of the dosage form through the GIT, it remains intact in the stomach and small intestine where very little microbially degradable activity is present that is quiet insufficient for cleavage of polymer coating. Release of the drugs from azo polymer coated formulation is supposed to take place after reduction and thus degradation of the azo bonds by the azo reductase enzymes released by the azo bacters present in the colonic microflora. Since the concept of this strategy is based on the metabolic activity of azo reductase produced by azo bacters of colon, the bacterial degradation of polymeric coating may be effected by several other factors e.g. dietary fermentation precursors, type of food consumed and coadministration of chemotherapeutic agents. Administration of antibiotics may result in the partial or complete destruction of colonic microflora, which adversely affect the release of bioactive agents. summarizes the synthesis and description of various azo polymers that have been exploited for colon targeted drug delivery.

Linear-type-segmented polyurethanes containing an azo group in the main chain have been synthesised as coating material.[147-149] Since this polymer was degraded specifically by the action of intestinal flora, the dosage form coated with this polymer would be effective for colon targeting oforally administered drugs. However, this poly-urathane based on m-xylene diisocynate (XDI), was soluble only in limited solvents and has been thought to be clinically inapplicable due to the trace amount of remaining solvent. Therefore Yamaoka et al[149] synthesised a segmented polyurethane containing azo aromatic groups in the main chain by ratio of isophorone diisocyanate with a mixture of m, m'-di (hydroxymethyl) azobenzene, poly (ethylene glycol), and 1, 2-propanediol. This polyurethane was soluble in various solvents and showed a good coating and film-forming property. A solution-cast film of this polyurethane was found to be degraded in a culture of intestinal flora with the azo group reduction to hydroazo groups, not to amino groups. The film degradation, therefore, was attributed to the decreased cohesive energy in the hydroazo polymer compared with that in the original azo polymer. Then, the drug pellets containing water-soluble drugs were undercoated with (carboxymethyl) (ethyl) -cellulose and over coated with the azo polymer in order to examine the drug-releasing profiles in the culture of intestinal flora.

The release rate of drugs from these double-coating pellets was found to depend on the molecular weight and the composition of the polyurethane used as the overcoat as well as the hydrophilicity of the incorporated drugs. Since the polyurethane was glassy and its segment motion or conformational change was frozen, the structure change should be retarded even after partial reduction of the azo groups, resulting in the effective prevention of the drug leakage. Further, Chavan et al[150] synthesised a urethane-based analogue containing an azo aromatic linkage in the backbone for use in colon-specific delivery systems by reacting toluene-2, 6-diisocyanate with a mixture of an aromatic azo diol, (bis-4-hydroxyphenyl)-4, 4' diazobiphenyl, poly (ethylene glycol) and 1, 2-propanediol (propylene glycol). The compounds exhibited low molecular weight, lacked film-forming properties and crystallinity in the structure. An *in vitro* bacterial degradation test to demonstrate the susceptibility of azo bond to bacterial enzymes was performed using media inoculated with lactobacillus culture. The results indicated degradation of films by azoreductase. *In vitro* permeation of 5-ASA was studied in control and lactobacilli-treated films. The permeability of the lactobacilli treated films was significantly increased suggesting the potential of these compounds for application in colonic targeting. Lehmann and Drehher[151] used a suspension of natural polygalactomannans in polymethacrylate solutions to form degradable coating. The polygalactomannans form a swellable layer around the drug core, thus delaying the release of drug in the small intestine. They are destroyed enzymatically in the colon and consequently the drug is released. A novel oral delivery system for the treatment of IBD based on the microencapsulation of anti-inflammatory drugs, sulfasalazine and betamethasone using different biodegradable polymers, poly (-caprolactone), polylactic acid and poly(lactic-co-glycolic acid), was prepared either by the water-in-oil-in-water (w/o/w) or the solid-in-oil-in-water (s/o/w) solvent evaporation method.[152] Microparticles were characterized for their size, morphology, encapsulation efficiency and drug release. *In vitro* release studies showed a controlled release of sulfasalazine and betamethasone from microparticles prepared by the s/o/w-method while a pronounced burst release of sulfasalazine was observed from microparticles prepared by the w/o/w method.

4.4.9 Embedding in matrices and hydrogels

The drug molecules are embedded in the polymer matrix. The polymers used for this technique should exhibit degradability in the colon for liberation of entrapped drug. Polysaccharides, the polymer of monosaccharides retains their integrity because they are resistant to the digestive action of gastrointestinal enzymes. The

matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine but once they reach in the colon, they are acted upon by the bacterial polysaccharidases and results in the degradation of the matrices. This family of natural polymers has an appeal to the area of drug delivery as it is comprised of polymers with a large number of derivatizable groups, a wide range of molecular weights, varying chemical compositions, and for the most part, a low toxicity and biodegradability, yet a high stability. The most favorable property of these materials is that they are already approved as pharmaceutical excipients. A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans and locust bean gum have been investigated for their use in colon targeted drug delivery systems. The most important fact in the development of polysaccharide derivatives for colon targeted drug delivery is the selection of a suitable biodegradable polysaccharide. As these polysaccharides are usually soluble in water, they must be made water insoluble by crosslinking or hydrophobic derivatisation. Very important is an optimal proportional of the hydrophobic and hydrophilic parts respectively and the number of free hydroxy groups in the polymeric molecule.

5. NOVEL APPROACHES FOR COLON TARGETING

5.1 Osmotic drug delivery

The drug release from this device is achieved through an orifice by osmotic pressure generated inside the device. Metronidazole based on osmotic drug delivery system is formulated, which consist of drug and osmogen (mannitol and fructose). The core is prepared by direct compression having coat with semipermiable membrane which is made by cellulose acetate, PEG400, guar gum, acetone or methanol with coating thickness of 90μm. It is further coated with enteric polymer eudragit S100. During transit through GIT system remain intact in stomach due to coating of enteric polymer, but dissolve in small intestinal pH and intestinal fluid enter into the tablet core react with osmogen which build up osmotic pressure. As it reaches to colon the guar gum which is pore former is degraded by colonic microflora forming pore and due to osmotic pressure core breakage results in drug release in colon.[153] The OROS-CT can be used to target the drug locally to the colon for the treatment of disease or to achieve systemic absorption that is otherwise unattainable.[154] The OROS-CT system can be single osmotic unit or may incorporate as many as 5-6 push-pull units ,[155] each 4mm in diameter, encapsulated with in a hard gelatin capsule . Each bilayer push pull unit contains an osmotic push layer and a drug layer, both surrounded by a semipermeable 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. As the unit enter the small intestine, the coating dissolve in this higher pH environment (pH >7), water enters the unit, causing the osmotic push compartment to swell and concomitantly creates a flowable gel in the drug compartment. Swelling of the osmotic push compartment forces drug gel out of the orifice at a rate precisely controlled by the rate of water transport through the semipermeable membrane. For treating ulcerative colitis, each push pull unit is designed with a 3-4 hour post gastric delay to prevent drug delivery in the small intestine. Drug release begins when the unit reaches the colon. OROS-CT units can maintain a constant release rate for up to 24 h in the colon or can deliver drug over an internal as short as 4 h.[156]

5. 2 Gas Empowered Drug Delivery System (GEDD)

It is also a novel drug delivery system to colon which is designed to target the proteins and peptides to the intestinal region by using mucoadhesive polymer polyethylene oxide and TMC as penetration enhancer using CO_2. By the presence of mucoadhesive polymer the drug remains adhered to the mucous layer and the permeation enhancer is used to open the tight junctions to promote paracellular pathway for drug absorption. In this system the CO_2 gas is used as driving force to push the drug substance to the absorbing membrane and also it covers the dosage form completely to protect it from enzymatic and proteolytic degradation. CO_2 also functions as permeation enhancer by opening the tight junctions mechanically. This system is successful in delivering the drug to the intestine because of the use of CAP (cellulose acetate phthalate) which protects the dosage form from the acidic pH of stomach.[157]

5.3 Enterion capsule Technology

It is a 32-mm long, round-ended capsule and contains a drug reservoir with a volume capacity of approximately 1 ml. The capsule can be loaded with either a liquid formulation (e.g. Solution, Suspension) or a particulate formulation (e.g., powder, pellets) through an opening 9 mm in diameter, which is then sealed by inserting a push-on Cap fitted with a silicone O-ring. The floor of the drug reservoir is the piston face, which is held back against a compressed spring by a high tensile strength polymer filament. A radioactive marker is placed inside a separate sealed tracer port to allow real time visualization of the capsule location using the imaging technique of gamma Scintigraphy. When the capsule reaches the target location in the gastrointestinal tract, the contents are actively ejected by the external application of an oscillating magnetic field. The frequency of the magnetic field is set in the low MHz region so there is negligible absorption in body tissue but sufficiently high to induce drug release. The power induced in the coil by the magnetic field is fed to a tiny heater resistor located within a separate sealed electronics compartment inside the

capsule. The small size of the heater (less than 1mm) means that heat build up is extremely rapid. The heater resistor is in direct contact with the restraining filament, causing it to softer and breaks with the increase in temperature. This is turn, releases the spring and driver the piston. The resulting increase in pressure within the drug reservoir forces off the O-ring sealed cap and rapidly ejects the drug or drug formulation into the surrounding GI fluids. The piston motion is stopped near the end of the capsule, which maintains a seal and presents contact of the internal electronic compartments with the GI fluids. The movement of the piston also operates a switch, which directs some of the electrical energy away from the heater and uses it to transmit a weak radio signal at a precise frequency. Detection of this signal externally confirms that the capsule has opened successfully.[158-160]

5.4 Redox-sensitive polymers

Analogues to azo bond cleavage by intestinal enzymes, novel polymers that hydrolyzed nonenzymatically by enzymatically generated flavins are being developed for colon targeting. Biodegradation of azo polymers has been extensively studied in the literature[161,162] 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. Molecular modeling of low molecular weight azo compounds revealed that reduction of the azo bond to the hydroazo intermediate requires a low electron density within the azo region, and thus substitution of electron-withdrawing groups will favor this reaction. Redox potential is an expression of the total metabolic and bacterial activity in the colon and it is believed to be insensitive to dietary changes. The mean redox potential in proximal small bowl is - 67 ± 90 mv, in the distal small bowl is -196 ± 97 mv and in the colon is -145 ± 72 mv. Thus, microflorainduced changes in the redox potential can be used as a highly selective mechanism for targeting to the colon. Bragger et al.[163] carried out investigations into the azo reducing activity, which could 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 (E1/2 -470 mV) was reduced at the fastest rate of 0.75 mol l *-1* h *-1*, amaranth (E1/2 -568 mV) at 0.30 mol l-1 h *-1*, Orange II (E1/2 -648 mV) at 0.2 mol l *-1* h *-1* and tartrazine (E1/2 -700 mV) at 0.08 mol l-1 h-1. Similar observations were made with another colonic bacterium *Eubacterium limosum* . Disulphide compounds can also undergo degradation due to the influence of redox potential in the colon.[164] Noncrosslinked redox-sensitive polymers containing an azo and/or a disulphide linkage in the backbone have been synthesised.[165] Radiological studies in dogs have investigated *in vitro* behaviour of new polyurethane systems containing azo bonds .[166,167]

5.5 Bioadhesive systems

Oral administration of some drugs requires high local concentration in the large intestine for optimum therapeutic effects. Dissolution of dosage form and simultaneous absorption from upper GIT lead to low intracolonic drug concentration as well as absorption of drugs result in the generation of side effects. Bioadhesion is a process by which a dosage form remains in contact with particular organ for an augmented period of time. This longer residence time of drug would have high local concentration or improved absorption characteristics in case of poorly absorbable drugs. This strategy can be applied for the formulation of colonic drug delivery systems. Various polymers including polycarbophils, polyurethanes, polyethylene oxide and polypropyline oxide copolymers have been investigated as materials for bioadhesive systems.[168,169] Bioadhesion has been proposed as a means of improving the performance and extending the mean residence time of colonic drug delivery systems. [170,171] *In vitro* bioadhesion has been confirmed from many studies and few reports are available in the literature regarding the in vivo bioadhesion studies.[172,173] Kakoulides et al. [174,175] synthesized Azo-networks based on an acrylic backbone crosslinked with DVAB . The chemical structure of the synthesised series of copolymers was examined by infrared spectroscopy and nuclear magnetic resonance data. The thermal properties of the materials were assessed using a combination of thermal analysis techniques and their swelling behaviour was evaluated at physiologically relevant buffers designed to mimic the gastrointestinal environment. These networks were subjected to *in vitro* degradation and mucoadhesion (before and after degradation) testing in order to model their performance in the gastrointestinal tract. Advanced surface characterisation techniques (SEM, AFM, FTIR microscopy) were used to examine the network morphology prior to, and after degradation. These studies indicate that there is an optimum crosslinking density to allow non-adhesive particles to reach the colon. Within the colonic environment, the azo network degrades to produce a structure capable of developing mucoadhesive interactions with the colonic mucosa. Amino acids and polymers have used as drug carriers for colon targeted delivery of 5-ASA.[176] The used bioadhesive polymers such as HPMA copolymers that are used to mimic bioadhesive process occurring in the guinea pig GIT which are based on the presence of lectin like structures on enterocytes and in the mucus gel layer.[177] A water-soluble polymer containing salicylate residues azo-linked at the 5-position to the polymer backbone was synthesized for the treatment of IBD.[178] The design of targetable water-soluble polymeric drug carriers based on N- (2-hydroxy propyl) methacrylamide (HPMA) copolymers was described by Kopecek.[179]

6. EVALUATION OF COLON DRUG DELIVERY SYSTEMS (CDDS)

A successful colon targeted drug delivery system is one that don't release drug in the stomach and small intestine, but releases the drug in the colonic region. Different *in vitro* and *in vivo* methods are used to evaluate the colon targeted drug delivery.

6.1 *In vitro* evaluation

No standard evaluation technique is available for evaluation of CDDS as an ideal *in vitro* model due to variable conditions of gastro-intestinal tract such as pH, volume, stirring, bacteria, enzymes, enzyme activity and components of food. These conditions are influenced by diet & physical stress. The evaluation of colon targeted drug delivery systems includes the *in-vitro* dissolution study & *in vitro* enzymatic test.

6.1.1 In vitro dissolution test

The dissolution testing is done using the conventional basket method. The dissolution testing is done in different buffers to characterize the behaviour of formulations at different pH levels. The different media that are used for the dissolution testing of colon targeted drug delivery are pH 1.2 to simulate gastric fluid, pH 6.8 to simulate small intestine, pH 7.4 to simulate large intestine. The colon targeted drug delivery systems are tested for 2hr in 0.1N HCl, 3hr in pH 6.8 phosphate buffer and finally at pH 7.4 phosphate buffer. Buffers of the above pH are prepared to evaluate the colon targeted drug delivery systems.

6.1.2 In vitro enzymatic test

There are two tests for the evaluation of in-vitro enzymatic activity. (a) The carrier drug system is incubated in fermentor containing suitable medium for bacteria. The amount of drug released at different time intervals is determined. (b) Drug release study is performed in buffer medium containing enzymes pectinase, dextranase or rat or guinea pig or rabbit cecal contents. The amount of drug released in a particular time is directly proportional to rate of degradation of polymer carrier. In vitro enzymatic dissolution study of tablet made by natural guar gum and xanthan gum has been carried out in presence of galactomannase enzyme and in both presence and absence of caecal content[180,181]

In vitro evaluation studies were conducted for chitosan-containing microparticulate system for colon drug delivery. In this study fluorescin isothiocyanate-labelled bovine serum albumin (FITCBSA) was used as a model drug. The chitosan hydrogel beads which containing trypolyphosphte as counter ion. The protein release experiments were carried out in vitro under different conditions to simulate the pH and times likely to be encountered drug intestinal transit to the colon. Release of FITC-BSA from the chitosan beads was studied in sealed 25ml conical flasks at constant temperature in shaker bath at 370C and 60 SPM. Enzymatic degradations of chitosan by pancreatin and by pancreatic lipase present in simulated intestinal fluid were studied using a viscometric procedure.[182] The pharmacokinetic evaluation of guar gum based colon targeted tablets of mebendazole against an immediate release tablet was carried out in human volunteers. Six healthy volunteers participated in the study and a crossover design was followed. In this study, on oral administration of colon-targeted tablets mebendazole started appearance in the plasma in five hours and reached the peak plasma concentration at 9.4 ± 1.7 hrs (Tmax) where as the immediate release tablets produced at 3.4 ± 0.9 hrs (Tmax). The results of the study indicated that the guar gum based colon targeted tablets of mebendazole did not release the drug in stomach and small intestine, but delivered the drug to the colon resulting in a slow absorption of the drug and making the drug available for local action in colon.[183]

6.2 *In vivo* evaluation

The *in vivo* evaluation of the CDDS is done in dogs, guinea pigs, rats & pigs as they resemble the anatomic physiological conditions and microflora of human GIT. The distribution of various enzymes in gastro-intestinal tract of rat and rabbit is comparable to that in human. Techniques which are used for monitoring the invivo behaviour of colon targeted drug delivery are Stirring technique, Endoscopy, Radiotelemetry, Roentegenography and Gamma scintigraphy.

6.2.1 Gamma- scintigraphy

Gamma-saintigraphy is an image modality which enables the in vivo performance of drug delivery system to be visualized under normal physiological conditions in a non invasive manner. Through scinitigraphy imaging, the following information regarding the performance of a colon specific drug delivery system within human Gastro-intestinal tract can be obtained which are the location as a function of time of complete system disintegration, the extent of dispersion, the colon arrival time, stomach residence and small intestine transit time. Studies has been carried out in human subjects using technetium-99m-DTPA as tracing agent in sodium chloride core tablet and compression coated with guar gum act as protecting coat against upper Gastro-intestinal tract environment, and it has been observed that the tablet remain intact in stomach and intestinal pH but as it enter in ascending colon it gets degraded by colonic microflora and the releases drug.[184]

6.2.2 Roentgenography

This technique involve incorporation of radio opaque material instead of drug such as barium sulfate, which visualized by taking X- rays of abdomen after oral administration. It is possible to observe movement, location and the integrity of the dosages after oral administration by placing the subject under a fluoroscope and taking a series of X-rays at various time intervals.[185]

6.2.3 High frequency capsule

Colonoscopy and incubation are the techniques mostly used for the analysis of dosage form inside the body. High

frequency capsules are the smooth plastic capsules taken orally. These contain small latex balloon, drug and radiotracer substance. The drug and radiotracer are released by an impulse, and the release is analyzed inside the different parts of GIT. By this technique the absorption properties of drugs in the colon are monitored.[186-188]

7. CONCLUSION

The colonic region of the gastro-intestinal tract has become an increasingly important site for drug delivery and absorption. CDDS offers considerable therapeutic benefits to patients in terms of both local and systemic treatment. Colon specificity more likely to be achieved with systems that utilize natural materials that are degraded by colonic bacterial enzymes. Now several approaches have been investigated to achieve site specificity to colon. The selection of suitable carrier and/or coating system is a critical parameter in the fabrication of colon specific drug delivery. The recent advances in CDDS promotes targeting of drugs and peptides in the treatment and management of major diseases and infections of the colon.

REFERENCES

1. Sarasija S, Hota A. Colon-specific drug delivery systems. Ind J Pharm Sci 2000; 62: 1-8.
2. Patel G , Patel Gayatri C, Patel Ritesh B. Oral colon-specific drug delivery: a overview. Drug Delivery Technology 2006; 6(7): 62-71.
3. Chourasia MK, Jain SK. Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharmaceut Sci 2003; 6(1): 33-66.
4. Etienne Schacht, An Gevaert, El Refaie Kenawy, Polymers for colon-specific drug delivery. Journal of Controlled Release 1996; 39: 327-338.
5. Vyas SP , Khar Roop K. Systems for colon specific drug delivery. In: Controlled drug delivery concepts and advances 2006 ; 1st ed., Delhi, p.218-256.
6. Breimer DD. Future challenges for drug delivery. Journal of Controlled Release 1999; 62: 3–6.
7. Asghar LFA, Chandran S. Multiparticulat Formulation. Approach to Colon Specific Drug Delivery: Current Perspectives. Journal of Pharma Pharmceutical Sciences 2006; 9(3): 327-328.
8. Aurora J., Talwar N., Pathak V. Colonic drug delivery challenges and opportunities – an overview. European Gastroenterology Review 2006; 1-6.
9. Gaurav Tiwari1, Ruchi Tiwari, Pranay Wal, Ankita Wal, Awani K. Rai. A review on Primary and novel approaches for colon targeted drug delivery. IJD 2010;2: 01-11
10. Hovgaard L, Brøndsted L. Dextran hydrogels for colon-specific drug delivery. Journal of Controlled Release 1995;36 :12: 3-19.
11. Gerard J. Tortora, Bryan Derricksion. Principles of Anatomy and Physiology. 12th ed :959-960.
12. Malik K, Goswami L, Kothiyal P, Mukhopadhyay S. Review on Colon targeting Drug Delivery System: Novel Approaches, Anatomy and Evaluation. The Pharma Innovation 2012;1(9):1-12.
13. Brahmankar DM., Jaiswal Sunil B. Biopharmaceutics and Pharmacokinetics A treatise second edition 2009 ; 27-51, 67-71.
14. Verma S, Saini S, Kaul M, Rawat A. Colon Disease and Approaches: An Overview. IJPT 2011;3(3):1197-1213
15. Sheth Anish A, Walter M.D , Martin H, Floch MD. Diverticular disease and Diverticulitis. Am J Gastroenterol 2008; 103:1550–1556.
16. Gangurde H. H, Chordiya M. A, Tamizharasi S, Sivakumar T. Diseases, approaches and evaluation parameters for colon specific drug delivery. A review. Int. J. Drug Res. Tech 2012; 2:3: 239-262.
17. Salve PS. Development and in vitro evaluation colon targeted drug delivery system using natural gums. Asian J. pharma. Res 2011;1:4: 91-101.
18. David R, Friend. New oral delivery systems for treatment of inflammatory bowel disease. Advance Drug Delivery Reviews 2005; 57:2: 247-265.
19. Patel Z, Patel MR, Patel RR, Patel NM. Novel Technique: A Overview of Colon Targeted Drug Delivery System. Asian Journal of Pharmaceutical Science and research 2013;3:5:31-55.
20. Balamuralidhara V, Pramodkumar T.M, Srujana N, Venketesh M.P, Gupta NV, Karishna KL, Gangadharapp HV. pH Sensitive Drug Delivery System: A Review. Amrican Journal of Drug.Discovery and Development. 2011; 1:1:24-28.

21. Zhang S Q, Thumma S, Chen G H, Deng W B, Repka M A , San-Ming Li. *In vitro* and *in vivo* evaluation of tegaserod maleate pH-dependent tablets. European Journal of Pharmaceutics and Biopharmaceutics 2008; 69:247–254.
22. Khan, M.Z.I. Dissolution testing for sustained or controlled release oral dosage forms and correlation with in vivo date: challenges and opportunities. Int J Pharm1996; 140:141- 143.
23. Dressman J.B, Amidon C, Reppas C, Shah VP. Dissolution testing as prognostic tool for oral drug absorption: Immediate release dosage forms, Pharm Res 1998; 15: 11- 22.
24. Spitael J, Kinget R. Solubility and dissolution rate of enteric polymers. Acta Pharm Technol 1975; 25:163-168.
25. Spitael J, Kinget R. Factors affecting the dissolution rate of enteric coatings. Pharm Ind 1977; 39: 502-505.
26. Spitael J. Kinget R, Naessens K. Dissolution rate of cellulose acetate phthalate and Bronsted catalysis. Pharm Ind1980; 42: 846-849.
27. Spitael J, Kinget R. Influence of solvent composition upon film-coating. Pharm Acta Helv 1980; 55: 157-160.
28. Touitou E, Rubinstein A.Targeted eternal delivery of insulin to rats. Int J Pharm 1986; 30: 95-99.
29. Thomos P, Richards D, Richards A. Absorption of delayed release prednisolone in ulcerative colitis and Chron's disease. J Pharm Pharmacol 1985; 37: 757-758.
30. Van Saene JJM, Van Saene HFK, Geitz JN, Tarko-Smit NJP, Lerk CF. Quinolones and colonization resistance in human volunteers, Pharm Weekbl Sci Ed.1986; 8: 67- 71.
31. Khan A, Piris T J. An experiment to determine the active Therapeutic moiety of sulphasalazine. Lancet 1977; 2: 892-895.
32. Bogentoft C, Eskilsson C, Jonsson UE, Lagerstorm PO, Lovgren K RL. Delivery of drug to the colon by means of a new microencapsulated oral dosage form. Acta Phrarm Suec1 983; 20: 311-314.
33. Watkinson G. Sulphasalazine: A review of 40 years experience. Drugs 1986; 32(suppl 90. 1): 1-11.
34. Riley S A, Lecarpentier J, Mani V, Goodman M J, Mandal BK, Turnberg LA. Sulphasalazine induced seminal abnormalities in ulcerative colitis: Results of mesalazine substitute. Gut 1987; 28: 1008-101.
35. Al Mardini H, Lindsay D C, Deighton CM, Record C A. Effect of polymer coating on faecal recovery of ingested 5-aminosalicylic acid in patients with ulcerative colitis. Gut 1987; 28: 1084-1089.
36. Levine D S, Raisys A V. Coating of oral beclomethasone dipropionate capsules with cellulose acetate phthalate enhances delivery of topically active anti inflammatory drug to the terminal ileum. Gastroenterology 1987; 92: 1037-1044.
37. Hardy J G, Evans D F, Zaki I, Clark AG, Tonnesen HH, Gamst ON. Evaluation of an enteric coated naproxen tablet using gamma scintigraphy and pH monitoring. Int J Pharm 1987; 37: 245-250.
38. Khan M Z, Prebeg Z, Kurjakovic N. A pH-dependent colon targeted oral drug delivery system using methacrylic acid copolymers I. Manipulation of drug release using Eudragit® L100-55 and Eudragit® S100 combinations. J Control Rel 1999; 58: 215-22.
39. Lorenzo-Lamosa M L, Remunan-Lopez C, Vila-Jato J L, Alonso M J. Design of microencapsulated chitosan microspheres for colonic drug delivery. J Control Rel1998; 52:109-118.
40. Dew MJ, Ryder REJ, Evans N, Evans BK, Rhodes J. Colonic release of 5-aminosalicylic acid from an oral preparation in active ulcerative colitis. Br J Clin Pharmcol 1983; 16: 185-187.
41. Dew M J, Ebden P, Kidwai S, Lee G, Evans B K, Rhodes J. Comparison of the absorption and metabolism of sulphasalazine and acrylic-coated 5-aminosalicylic acid in normal subjects and in patients with colitis. Br J Clin Pharmcol 1984; 17: 474-476.
42. Hardy J G, Healy J N C, Reynolds J R. Evaluation of an enteric coated delayed release 5-aminosalicylic acid tablet in patients with inflammatory bowl disease. Aliment Pharmacol Ther 1987; 1:273-280.
43. Schroeder KW, Tremaine Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mild to moderately active ulcerative colitis. N Engl J Med 1987; 317: 1625-1629.
44. Norlander B, Gotthard R, Storm M. Pharmacokinetics of 5-aminosalicylic acid enteric-coated tablet and suppository dosage form. Aliment Pharmacol Ther 1989; 3: 333-342.

45. Jarnerot G. Newer 5-aminosalicylic acid based drugs in chronic inflammatory bowel disease. Drugs 1989;37: 73-86.

46. Faber SM , Korelitz BI. Experience with Eudragit-S-Coated mesalamine (Asacol) in inflammatory bowl disease: An open study. J Clin Gastroenterol 1993;17: 213-218.

47. Peeters R, Kinget R. Film-forming polymers for colonic drug delivery. I. Synthesis and physical and chemical properties of methyl derivatives of Eudragit S. Int J Pharm1993; 94: 125-134.

48. Rodríguez M, Vila-Jato J L, Torres D. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. J Control Rel. 1998; 55: 67-77.

49. Ashford M, Fell J T, Attwood D, Sharma H, Woodhead P J. An in vivo investigation into the suitability of pH-dependent polymers for colonic targeting. Int J Pharm1993; 95: 193-199.

50. Rodriguez M, Antúnez J A, Taboada C, Seijo B, Torres D. Colon-specific delivery of Budesonide from microencapsulated cellulosic cores: evaluation of the efficacy against colonic inflammation in rats. J Pharm Pharmacol 2001; 53: 1207-15.

51. Markus W, Rudolph K, Beckert S, Petereit H, Dressman J B. A new aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis Eur J Pharm Biopharm. 2001; 51: 183-190.

52. Brigitte S, Markus R, Gerhard R. In-vivo evaluation of EUDRACOLTM, A novel pH and time controlled multiple unit colonic drug delivery systems. Eudracol Abstract Final doc: CS-1; 2003.

53. Gang C, Feng A, Mei-Juan Z. Time and pH dependent colon specific drug delivery for orally administered diclofencac sodium and 5-amino salicylic acid. World J Gastroenterol 2004; 10(12): 1769-1774.

54. Mahkam M. New pH-sensitive glycopolymers for colon-specific drug delivery. Drug Delivery 2007; 14(3): 147-153.

55. Krogars K, Heinamaki J, Vesalahti J, Marvola M, Antikainen O, Yliruusi J, Marvola Yliruusi J. Extrusion-spheronization of pH-sensitive polymeric matrix pellets for possible colonic drug delivery. Int J Pharm 2000;199: 187-94.

56. Nykanen P, Krogars K, Sakkinen M, Heinamaki J, Jurjensson H, Veski P, Marvola M. Organic acids as excipients in matrix granules for colon-specific drug delivery. Int J Pharm 1999; 184:251-61.

57. Modasiya M, Patel K, Vishnu M. Pulsatile Drug Delivery System for Colon. International Journal of Research in Pharmaceutical and Biomedical Science. 2011; 2:3: 934-941.

58. Challa T, Vynala V, Allam K V. Colon Specific Drug Delivery Systems: A Review on Primary and Novel Approaches. International Journal of Pharmaceutical Sciences Review and Research. 2011; 7(2):171-181.

59. Kumar KVV, Kumar T S, Mani T T. Colon targeting drug delivery system: A review on recent approaches. International Journal Pharm Biomedical Sciences. 2011; 2(1): 11- 19.

60. Rangasamy M. Colon Targeted Drug Delivery Systems: A Review. International Journal of Drug Formulation & Research. 2010; 1(2): 30-54.

61. Wasnik S, Parmar P. The Design of Colon-Specific Drug Delivery System and Different Approaches to Treat Colon Disease. International Journal of Pharmaceutical Sciences Review and Research. 2011; 6(2): 167-177.

62. Sinha VR, Bhinge JR, Kumaria R. Development of pulsatile systems for targeted drug delivery of celicoxib for prophylaxis of colorectal cancer Drug Delivery 2006; 13: 221- 225.

63. Wu B, Shun N, Wei X . Characterization of 5-fluorouracil release from hydroxy propyl methyl cellulose compression-coated tablets. Pharm Dev Technol 2007;12(2): 203 210.

64. Gazzaniga A, Bussetti C, Moro L, Sangali M E, Giordano F. Time dependent oral delivery system for colon targeting. STP Pharm Sci 1995; 5: 83-88.

65. Gupta VK, Beckert TE, Price JC. A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. Int J Pharm 2001; 213: 1-2:83 - 91.

66. Ishibashi T, Hatano H, Kobayashi M, Mizobe M, Yoshino H. Design and evaluation of a new capsule-type dosage form for colon-targeted delivery of drugs. Int J Pharm1998; 168: 31-40.

67. Fukui E, Miyamura N, Uemura K, Kobayashi M. Preparation of enteric coated timed release press-coated tablets and evaluation of

their function by in vitro and in vivo tests for colon targeting .Int J Pharm 2000; 204: 7 - 15.

68. Fukui E, Miyamura N, Kobayashi M. An in vitro investigation of the suitability of presscoated tablets with hydroxypropylmethylcellulose acetate succinate (HPMCAS) and hydrophobic additives in the outer shell for colon targeting. J Control Rel 2001; 70: 97-107.
69. Binns J S, Stevens H NE, Bakhashaer M, Wilson G G. Colon targeted release using the Pulsincap delviery system. Int Symp Controlled Rel Bioact Mater 1994; 21: 260-261.
70. MacNeil M E, Rashid, Stevens H N E. Dispensing device. World Patent. WO 9009168;1990.
71. Gupta VK, Assmus MW, Beckert TE, Price JC. A novel pH- and time-based multi-unit potential colonic drug delivery system. II. Optimization of multiple response variables. Int J Pharm 2001; 213: 93-102.
72. Pozzi F, Furlani P, Gazzaiga A, Daviss S S, Wilding I R. A new oral dosage form for fast and complex release of drug after a predetermined lag time. J Control Rel. 1994; 31: 99-104.
73. Niwa K, Takaya T, Morimoto T, Takada I. Preparation and evaluation of a time-controlled release capsule made of ethyl cellulose for colon delivery of drugs. J Drug Target 1995; 3: 83-89.
74. Sharma A, Jain K A. Colon Targeted Drug Delivery Using Different Approaches. International Journal of Pharmaceutical Studies and Research 2010; 1(1): 60
75. Koteshwara K B , Naha A, Nampoothiri M. Colon Targeted Drug Delivery System: A Review. International Journal of Research in Ayurveda & Pharmacy. 2011; 2(1): 60-65.
76. Kothawade PD, Gangurde HH, Surawase RK, Wagh MA, Tamizharasi S. Conventional and Novel Approaches for Colon Specific Drug Delivery: A Review. Journal of Science & Technology 2011; 6(2): 33-56
77. Muraoka M, Kimura G, Zhaopeng H, Takada K. Ulcerative colitis—colon delivery of 5-aminosalicylic acid. Nippon Rinsho 1998;56: 788-794.
78. Takaya T, Niwa K, Muraoka M, Ogita I, Nagai N, Yano R, Kimura G, YoshikawaY, Yoshikawa H, Takada K. Importance of dissolution process on systemic availability of drugs delivered by colon delivery system. J Control Rel. 1998; 50: 111-122.
79. Muraoka M, Hu Z, Shimokawa T, Sekino S, Kurogoshi R, Kuboi Y, Yoshikawa Y, Takada K. Evaluation of intestinal pressure-controlled colon delivery capsule containing caffeine as a model drug in human volunteers. J Control Rel. 1998; 52: 119-129.
80. Libio Yang, James S, Chu Joseph A. Colon-specific drug delivery: new approaches and in vitro / in vivo evaluation. International Journal of Pharmaceutics 2002;235: 1-15.
81. Masataka K, Shunsuke W, Hitoshi K. Studies on lactulose formulations for colon- specific drug delivery. International Journal of Pharmaceutics 2002; 249: 33-43.
82. Orienti I, Trere R , Zecchi V. Hydrogels formed by cross-linked polyvinyl alcohol as colon- specific drug delivery systems. Drug development and Industrial Pharmacy. 2001;27(8): 877-884.
83. Krishnaiah YSR, Veer Raju P, Dinesh Kumar B. Development of colon targeted drug delivery systems for Mebendazole. Journal of controlled Release 2001; 77: 87- 95.
84. Krishnaiah YSR, Veer Raju ., Dinesh Kumar B . Pharmacokinetic evaluation of guar gum-based colon targeted drug delivery systems of Mebendazole in healthy volunteers. Journal of controlled Release 2003; 88: 95-103.
85. Raffi R, Franklin W, Cerniglia C E. Azoreductase activity of anaerobic bacteria isolated from human intestinal microflora. Appl Environ Mivrobiol 1990; 56: 2146-2151.
86. Raffi R, Franklin W, Heflich R H, Cerniglia C E. Reduction of nitroaromatic compounds by anaerobic bacteria isolated from the human intestinal tract. Appl Environ Mivrobiol 1991; 57: 962-968.
87. Khan A, Truelove S C, Aronseq J K. The disposition and metabolism of sulphasalazine (salicylazosulphapyridine) in man. Br J Clin Pharmacol 1982; 13: 523-528.
88. Chan R P, Pope D J, Gilbert A P B, Lennard-Jones J P.Studies of two novel sulfaslazine analogue: ipsalazine and balsalazine. Dig Dis Sci 1983; 28: 609-615.
89. Hartalsky A. Salicylazobenzoic acid in ulcerative colitis. Lancet .1982; 1: 960.

90. Garreto M , Ridell R H, Wurans C S. Treatment of chronic ulcerative colitis with poly ASA: A new nonabosrbable carrier for release of 5-aminosalicylic acid in the colon. Gastroenterology 1981; 84: 1162.

91. Willoughby C P, Aronson J K, Agback H, Bodin N O, Anderson E, Truelove SC. Disposition in normal volunteers of sodium azodisalicylate, a potential therapeutic agent in inflammatory bowl disease. Gut 1981; 22: A431.

92. Willoughby C P , Aronson J K, Agback H, Bodin, N O, Anderson E , Truelove SC. Disposition in normal volunteers of sodium azodisalicylate, a potential therapeutic agent in ulcerative colitis. Gut 1982; 23: 1081-1087.

93. Lauristein K, Hansen J , Ryde H M , Rask-Madsen J. Colonic azodisalicylate metabolism determined by in vivo dialysis in healthy volunteers and patients with ulcerative colitis. Gastroenterology 1984; 86: 1496-1500, 1984.

94. Sandberg-Gertzen H, Ryde H M, Janerot G. Absorption, metabolism and excretion of a single 1 g dose of azodisal sodium in subjects with ileostom. Scan J Gastroenterol.1983;88: 107-111.

95. Van Hogezand R A, Van Hees P A M, Zwaneburg B, Van Rossum J M, Van Tongeren J H M. Disposition of sodium azodisalicylate in healthy subjects, Gastroenterolgy 1985; 88: 717-722.

96. Rao S S C, Read N W, Holdsworth C D. Influence of olsalazine on gastrointestinal transit in ulcerative colitis. Gut 1987; 28: 1474-1477.

97. Pamucku R, Hanauer S, Chang E B. Effect of disodium azosalicylate on electrolyte transport in the rabbit ileum and colon in vitro. Gastroenterology 1988; 95: 975-981.

98. McIntyre P B, Rodrigues C A , Lennard-Jones J E. Balsalazine in the maintenance treatment of patients with ulcerative colitis, a double-blind comparison with sulphasalazine. Aliment Pharmacol Ther 1889; 2: 237-243.

99. Riley SA, Turnberg LA. Sulphasalazine and the aminosalicylates in the treatment of inflammatory bowl disease. Q J Med 1990; 75: 561-562.

100. Sakuma S, Lu Zheng-Rong, Kopeková P , Kopeek J. Biorecognizable HPMA copolymer-drug conjugates for colon-specific delivery of 9-aminocamptothecin. J Control Rel 2001; 75: 365-379.

101. Friend D R. Glycosides in colonic drug delivery. In: Friend, D.R. (Ed). Oral colon specific drug delivery. CRC Press, Boca Raton, 1992; 153-187.

102. Pownall J H , Hickson D L, Smith L C.Transport of Biological Lipophils: Effect of Lipophilic structure. J Am Chem Soc 1983;105: 2440-2445.

103. Kobashi K , Nishimura T , Kusaka M , Hottari M, Namba T. Metabolism of sennoids by human intetinal bacteria. J Med Plant Res 1980 ;40: 225-236.

104. Conchie J , Macleod D C. Glycosidase in mammalian alimentary tract. Nature 1959; 184: 1233.

105. Rubinstein A. Approaches and opportunities in colon-specific drug delivery. Crit Rev Ther Carr Syst 1995; 12: 101-149.

106. Friend D R, Chang G W A. colon-specific drug delivery based on the drug glycosidases of colonic bacteria. J Med Chem 1984; 27: 261-266.

107. Friend D R, Chang G W. Drug Glycosides: Potential prodrug for colon-specific drug delivery.J Med Chem 1985; 85: 51-57.

108. Friend D R , Tozer T N. Drug glycoside in colon-specific drug delivery. J Control Rel 1992 ;19:109-120.

109. Friend D R , Philips S, McLeod A, Tozer T N. Relative anti-inflammatory effect of oral dexmamethasone-b-D-glucoside and dexamethasone in experimental inflammatory bowl disease in guinea pig. J Pharm Pharmacol 1991; 43: 353-355.

110. Minami K, Hiramaya F, Uekama K. Colon-specific drug delivery based on cyclodextrin prodrug: release behaviour of biphenyl acetic acid form its cyclodextrin conjugates in rat intestinal tract after oral administration. J Pharm Sci 1998; 87: 715- 720.

111. Scheline R.P. Drug metabolism by intestinal microorganism. J Pharm Sci 1968; 57: 32. 2021-2037.

112. Simpkins J W, Smulkowski M, Dixon R, Tuttle R. Evidence for the delivery of narcotic antagonists to the colon as their glucuronide conjugates. J Pharmacol Exp Ther 1988; 244: 195-205.

113. Haeberlin B, Rubas W, Nolen H W, Friend DR. In vitro evaluation of dexamethasoneb- D-glucuronide for colon-specific drug delivery. Pharm Res 1993;10:1553-1562.

114. Mcleod A D, Feclorak R N, Friend D R, Tozer T N, Cui N. A glucocorticoid prodrug facilitates normal mucosal function in rat colitis without adrenal suppression. Gastroenterology 1994; 106:405-413.

115. Cui NF, Fedorak RNA Budesonide prodrug accelerates treatment of colitis in rats. Gut 1994; 35: 1439-1446.

116. Fedorak R N, Haeberlin B , Empey L R. Colonic drug delivery of dexamethasone from a prodrug accelerates healing of colitis in rat without adrenal suppression. Gastroenterology 1995; 108:1688-1699.

117. Nolen HW, Fedorak RN , Friend DR. Steady-state pharmacokinetics of Corticosteroid delivery from glucuronide prodrugs in normal and colitic rat. Biopharm Drug Dispose 1997; 18: 681-695.

118. Loftsson T, Brewster ME, Derendorf H, Bodor N. 2-hydroxypropyl-b-cyclodextrin: properties and usage in pharmaceutical formulations. Pharm Ztg Wiss 1991; 4: 5-10.

119. Uekama K, Hiramaya F, Irie T. Pharmaceutical uses of cyclodextrin derivatives. In: (Szycher,M., Ed.). High performance Biomaterials, Technomic publishing, Lancaster, pp 1991;789-806.

120. Szejtli J, Szente L. Ed. Proceedings of the Eighth international symposium on cyclodextrins, Kluwer, Dordrecht, 1996.

121. Stella VJ, Rajewski RA. Cyclodextrins: their future in drug formulation and delivery. Pharm Res 1997; 14: 556-567.

122. AndersenG H, Robbins F M, Domingues F J, Morres R G, Long C L. The utilisation of schardinger dextrins by the rat. Toxicol Appl Pharmacol 1983; 5: 257-266.

123. Antenucci R , Palmer J K. Enzymatic degradation of a and b-cyclodextrins by bacteroids of the human colon. J Agric Food Chem 1984; 32: 1316-1321.

124. Gerloczy A, Fonagy A, Keresztes P, Perlaky L, Szejtli J. Absorption, distribution, excretion and metabolism of orally administered 14C-b-cyclodextrin in rat. Arzneim Forsch / Drug Research 1985;35:1042-1047.

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

126. Minami K, Hiramaya F, Uekama K. Colon-specific drug delivery based on a-cyclodextrin prodrug: release behaviour of biphenyl acetic acid from its cyclodextrin conjugates in rat intestinal tracts after oral administration. J Pharm Sci 1998; 87: 715-720.

127. Uekama K, Minami K, Hirayama F. 6A-O-[(4-biphenylyl)acetyl]-alpha-, -beta- and gamma-cyclodextrins and 6A-deoxy-6A-[[(4-biphenylyl)acetyl]amino]-alpha-, beta-, and -gammacyclodextrins: potential prodrugs for colon-specific delivery. J Med Chem 1997; 40:2755-61.

128. Hiramaya F, Minami K, Uekama K. In vitro evaluation of biphenyl acetic acid- betacyclodextrin conjugates as colon-targeting prodrugs: Drug release behaviour in rat biological media. J Pharm Pharmacol 1996; 48: 27-31.

129. Yano H, Hirayama F, Kamada M, Arima H, Uekama K, Uekama K. Colon specific delivery of prednisolone-appended alpha-cyclodextrin conjugate: alleviation of systemic side effect after oral administration. J Control Rel 2002; 79: 103-12.

130. Frank DW, Gray JE, Weaver RN. Cyclodextrin nephrosis in the rat. Am J Pathol 1976; 83: 367-382.

131. Rajeskwi R A, Traiger G, Bresnahan A, Jaberaboansari P, Stella V J Thompson DO. Preliminary safety evaluation of parenterally administered sulfoalkyl ether b-cyclodextrin derivatives. J Pharm Sci 1995; 84: 927-932.

132. Uekama M, Horikawa T, Yamanaka M, Hiramaya F. Peracylated b-cyclodextrins as novel sustained release carries for a water soluble drug, molsidomine. J Pharm Pharmacol 1994; 46: 714-717.

133. Larsen C, Harboe E, Johansen M, Olesen H P. Macromoleculer prodrugs. XVI. Colon targeted delivery. Comparison of the rate of release of naproxen from dextran ester prodrug in homogenates of various segments of the pig gastrointestinal tract. Pharm Res 1989; 6: 995-999.

134. Harboe E, Larsen C, Johansen M, Olesen HP. Macromoleculer prodrugs. XV. Colon targeted delivery. Bioavailability of naproxen

from orally administered dextran ester prodrugs varying molecular size in the pig. Pharm Res 1989; 6: 919-923.

135. Harboe E, Larsen C, Johansen M, Olesen H P. Macromoleculer prodrugs. XIV. Absorption characteristics of naproxen after oral administration of a dextran T-70-naproxen ester prodrugs in pigs. Int J Pharm 1989; 53: 157-165.

136. Johansen M, Larsen C. Stability kinetics and of hydrolysis of metronidazole monosuccinate in aqueous solution and in plasma. Int J Pharm 1984; 21: 201-209.

137. Johansen M, Larsen C. A comparison of the chemical stability and the enzymatic hydrolysis of a series of aliphatic and aromatic ester derivatives of metronidazole. Int J Pharm 1985; 27: 219-231.

138. Vermeersch J, Vandoorne F, Permentier D, Schacht E. Macromoleculer prodrugs of metronidazole. Esterification of hydroxyl containing Polymers with metronidazole monosuccinate. Bull Soc Chim Belg 1985; 94: 591-596.

139. McLeod AD, Friend DR, Tozer TN. Synthesis and chemical stability of glucocorticoiddextran ester: Potential prodrugs for colon-specific drug delivery. Int J Pharm 1993; 92: 105-114.

140. Nakamura J, Asai K, Nishida K, Sasaki H. A novel prodrug of salicylic acid, salicylic acid-glutamic acid conjugate utilising hydrolysis in rabbit intestinal microorganism. Chem Pharm Bull 1992; 40: 2164-2168.

141. Nakamura J, Kido M, Nishida K, Sasaki H. Hydrolysis of salicylic acid-tyrosine salicylic acid-methionine prodrugs in rabbits. Int J Pharm 1992; 87: 59-66.

142. Nakamura J, Kido M, Nishida K, Sasaki H. Effect of oral pretreatment with ntibiotics on the hydrolysis of salicylic acid-tyrosine and salicylic acid-methionine prodrugs in rabbit intestinal microorganisms. Chem Pharm Bull 1992; 40: 2572-2576.

143. Nakamura J, Tagami K, Nishida K, Sasaki H. Unequal hydrolysis of salicylic acid- D-alanine and salicylic acid-L-alanine conjugate in rabbit intestinal microorganisms. Chem Pharm Bull 1992; 40: 547-549..

144. Schacht E, Callant D, Verstraete W. Synthesis and evaluation of polymeric prodrugs of 5-aminosalicylic acid. Proc Int Symp Contr Rel Bioact Materl 1991; 18: 686-687.

145. Leopold CS, Friend DR. *In vitro* study for the assessment of poly(L-Aspartic acid) as a drug carrier for colon-specific drug delivery. Int J Pharm1995; 126: 139-145.

146. Leopold CS, Friend DR. *In vivo* pharmacokinetics study for the assessment of poly(LAspartic acid) as a drug carrier for colon-specific drug delivery. J Pharmacokinet Biopharm 1995; 23: 397-406.

147. Kimura Y, Makita Y, Kumagi T, Yamane T, Kiato H, Sasatani H , Kim S I. Degradation of azo-containg polyurethane by the action of intestinal flora: its metabolism and application as a drug delivery system. Polymer 1992; 33: 5294-5299.

148. Ueda T, Yamaoka M, Miyamoto M, Kimura Y. Bacterial reduction of azo compounds as a model reaction for the degradation of azo-containing polyurethane by the action of intestinal flora. Bull Chem Soc Jpn 1996; 69: 1139-1142.

149. Yamaoka T, Makita Y, Sasatani H, Kim S I, Kimura Y. Linear type azo-containing polyurethane as drug-coating material for colon-specific delivery: its properties, degradation behaviour, and utilization for drug formulation. J Control Rel. 2000; 66: 187-97.

150. Chavan M S, Sant VP, Nagarsenker M S. Azo-containing urethane analogues for colonic drug delivery: synthesis, characterization and in-vitro evaluation. J Pharm Pharmacol 2001; 53: 895-900.

151. Lehmann KOR, Drehher. Methacrylated galactomannan coating for colon-specific drug delivery. Proc Int Symp Contrl Rel Bioact Mater 1991; 18: 331-332.

152. Lamprecht A, Torres H R, Schafer U, Lehr C M. Biodegradable microparticles as a two-drug controlled release formulation: a potential treatment of inflammatory bowel disease, J Control Rel 2000; 69: 445-454.

153. Kumar P , Singh S, Mishra B. Colon targeted delivery system of metronidazole based on osmotic technology, Chem. Pharm. Bull 2008; 56:9:234-1242.

154. Theeuwes F, Guittard G, Wong P. Delivery of drugs to colon by oral dosage forms. US Patent 4904474, 1990.

155. Swanson D, Barclay B, Wong P, Theeuwes F. Nifedipine gastro-intestinal therapeutics system. Am J Med 1987; 83 (suppl. 6B): 3.

156. Ghosh PK, Gupta VB, Gondoliya B, Rathore MS. Probiotic assisted colon specific delivery of diclofenac sodium from gur gum matrix tablet, in vitro evaluation. Asian J Pharm 2010; 4:4:173-178.

157. Development of a Gas Empowered Drug Delivery system for peptide delivery in the small intestine. J Control Release 2009; 134 (1):11-7.

158. Kumar Voleti V, Kumar Junju M, Rayaprolu M. Chronotherapeutic delivery of ibuprofen using chemically modified natural gum. International Journal of Pharmaceutical Research and life Science 2013;1:2:141-148.

159. The Enterion Capsule: A Novel Technology for Understanding the Biopharmaceutical Complexity of New Molecular Entities (NMEs) 2008;1.

160. Laroui H, Dalmasso G, Nguyen HT, Yan Y, Sitaraman SV, Merlin D. Drug-loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse model. Gastroenterology 2010; 138:3843-53.

161. Gingell R, Walker R. Mechanism of azo reduction by non-specificity of Streptococcus faecalis azo reductase. Xenobiotica1975; 5: 563-571.

162. Roxon JJ, Ryan AJ, wright SE. Enzymatic reduction of tartrazine by Proteus vulgaris from rats. Food Cosmet Toxicol 1967; 5: 645-656.

163. Bragger J L, Lloyd AW, Soozandehfar S H, Bloomfield S F, Marriott C, Martin GP. Investigations into the azo reducing activity of a common colonic microorganism. Int J Pharm1997; 157: 61-71.

164. Larsel, G L, Larson J P, Gustafsson J A. Cystein conjugate beta-lyase in the gastrointestinal bacterium Fusobacterium necrophorum. Xenobiotica 1983 13: 687-693.

165. Schacht E, Wilding I R. Process for the preparation of azo- and/or disulfide-containing polymers. WO 9111175.

166. Kimura Y, Kim S, Nishiura A. Polyurethanes. European Patent. EP 0398472, 1991.

167. Kimura Y, Makita Y, Kumagai T. Degradation of azo-containing polyurethane by the action of intestinal flora: its mechanism and application as a drug delivery. Polymer. 1992; 33: 5294-5299.

168. Gurny R, Junginger HE. Bioadhesion-possibilities and future trends. Wissenchaftliche Verlag, Stuttgart, 1989.

169. Lenaerts V, Gurny R. Bioadhesive drug delivery systems. CRC Press, 221. Boca Raton 1990.

170. Chickering DE, Mathiowitz E. Bioadhesive microspheres.. A novel electrobalance-based method to study adhesive interactions between individual microspheres and intestinal mucosa. J Control Rel. 1995; 34: 251-262.

171. Chickering DE, Jacob JS. Mathiowitz E.,Bioadhesive microspheres. Characterisation and evaluation of bioadhesion involving hard, bioerodible polymers and soft-tissue. Reacting Polymers 1995; 25: 189-206.

172. Khosla R, Davis SS. The effect of polycarbophil on the gastric emptying of pellets. J Pharm Pharmacol 1987; 39: 47-49.

173. Haris D, Fell J T, Sharma H L, Taylor DC. GI transit of potential bioadhesive formulations in men: A scintigraphy study. J Control Rel 1990; 12: 45-53.

174. Kakoulides E P, Smart JD, Tsibouklis J. Azocross-linked poly(acrylic acid) for colonic delivery and adhesion specificity: synthesis and characterisation. J Control Rel 1998; 52: 291-300.

175. Kakoulides E P, Smart J D, Tsibouklis J. Azocrosslinked poly(acrylic acid) for colonic delivery and adhesion specificity: in vitro degradation and preliminary ex vivo bioadhesion studies. J Control Rel 1998; 54: 95-109.

176. Pellicciari R, Garzon-Aburbeh , Natalini B. Brush-border-enzyme-mediated intestine-specific drug delivery. Amino acid prodrugs of 5-aminosalicylic acid. J Med Chem1993; 36: 4201-4207.

177. Rihova B. Bioadhesive polymers for oral drug delivery. Adv Exp Med Biol 1995; 371B: 1491-1494.

178. Brown JP, McGarrough GV, Parkinson TM, Wingerd RE, Onderdonk AB. Polymeric drug for treatment of inflammatory bowl disease. J Med Chem1983; 26: 1300-1307.

179. Kopecek J. The potential of water-soluble polymeric carriers in targeted and site- specific drug delivery. J Control Rel 1990; 11: 279-290.

180. Koteshwara K B. Primary and novel approaches for colon targeted drug delivery. A review. Int. Journal of Research in Ayurvedic & Pharmacy.2011; 2:1:60-65.

181. Salve PS. Development and in vitro evaluation colon targeted drug delivery system using natural gums. Asian J. pharma. Res 2011;1:4: 91-101.

182. Hua Z, Ibrahim A, Alsarra, SHN .An *in vitro* evaluation of chitosancontaining multiparticulate system for macromolecule delivery to the colon. International Journal of Pharmaceutics 2002; 239:197-205.

183. Munjeri O, Collett JH, Fell JT. Hydrogel beads based on amidated pectins for colonspecific drug delivery: the role of Chitosan in modifying drug release. Journal of Controlled Release 1997; 46:273-278.

184. Nicholas M E, Panaganti S, Prabakaran L, Jayveera KN. Colon specific drug delivery system: A review. International Journal of Pharmaceutical Science and Research 2011; 2(10):2 545-561.

185. Gopinath H , Bhowmik D, Venugopal P. Shanmugasundaram S. Review on colon specific drug delivery strategies and in vitro & in vivo evaluation. Elixir pharmacy 2013; 57:13955-13963.

186. Takemura S, Watanabe S, Katsuma M, Fuku iM : Colon specific drug release system. U.S .Patent 1998; 09/183339.

187. Fatmanur T, Fusin A, Sevgi J. *In vitro* and *In-vivo* evaluation of mesalazine-guar gum matrix tablets for colonic drug delivery. Journal of Drug Targeting 2004; 12(2): 105-12.

188. Mine O, Erdal C, Ahmet A. Design and evaluation of colon-specific drug delivery system containing flubiprofen microsponges. International Journal of Pharmaceutics 2006; 318:103-117.