I. COLON TARGETED DRUG DELIVERY

Till date, oral delivery is still the preferred route of drug administration, especially for chronic therapies where repeated administration is required. Oral administration offers patients less pain, greater convenience, and higher likelihood of compliance and reduced risk of cross infections. Thus formulations of oral drug delivery continue to dominate more than half of the drug delivery market share. Despite these advantages, the oral route is not amenable to the administration of most protein and polypeptide drugs available today, due to their high susceptibility to digestive enzymes in the gastrointestinal tract (GIT), poor absorption, and their limited ability to transport across the intestinal epithelial barrier. As a result, new strategies of drug delivery have been developed to overcome obstacles encountered by oral delivery. Among these strategies, colon-specific delivery has been extensively studied from the last two decades (1).

The colonic region of the GIT is one area that would benefit from the development and use of such modified release technologies. Although considered by many colon is an innocuous organ that has simple functions in the form of water and electrolyte absorption, formation, storage and expulsion of fecal material, the colon is vulnerable to a number of disorders including inflammatory bowel disease (IBD), ulcerative colitis, Crohn’s disease, irritable bowel syndrome (IBS) and carcinomas (2). Targeted drug delivery to the colon, by means of combination of one or more controlled release dosage forms, hardly releases drug in the upper part of the GIT but rapidly releases in the colon following oral administration. Specifically delivering drugs to the colon leads to a lot of benefits which can be used in terms of improving safety and reducing toxicity when treating local or systemic chronic diseases (3).

Colon is an area where protein drugs are free from the attack of numerous protease, is thought to be an ideal location to direct the drugs into the blood stream and the immune system. Most of the conventional drug delivery systems for treating the colon disorders are failing, as the drugs do not reach the site of action in appropriate concentrations.
Thus, an effective and safe therapy of these colonic disorders, using site specific drug delivery system is a challenging task to the pharmaceutical technologists (4). Dosage forms that deliver drugs into the colon rather than upper GIT offers number of advantages (5, 6):

1. Drug delivery to colon is valuable in the treatment of diseases of colon whereby high local concentration can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT or unnecessary systemic absorption.
2. Colon Drug Delivery System (CDDS) would be useful when delay in drug absorption is desired from therapeutic point of view, such as treatment of disease that have high peak in the early morning like nocturnal asthma, angina, arthritis.
3. The colon is rich in lymphoid tissue, uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery.
4. Colon is a promising site for delivery of peptide and protein drugs because protein and peptide drugs get destroyed or inactivated in acidic environment of the stomach or by pancreatic enzymes in the small intestine.
5. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. This region of the colon is recognized as having somewhat less hostile environment with less diversity and intensity of activity than the stomach and small intestine. The colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs.

1.1 THERAPEUTIC APPLICATIONS IN COLON SPECIFIC DELIVERY:
Targeting drugs to the colon has proven quite valuable in a variety of disorders, and the colon has proven to be a potential site for local as well as systemic administration of drugs. Colon targeting has proven beneficial for (6):

1. Local action in a variety of disease conditions can be achieved.
2. Aminosalicylates, corticosteroids, immunosuppressants, cationized antioxidant enzymes, genetically engineered bacteria to produce cytokines, nicotine, and
other drugs have exhibited significantly enhanced efficacy when delivered to the colon.

3. Targeting drugs to cancer cells through receptors and ligands have opened up new avenues in the treatment of colonic cancer.

4. Colon targeting has also proven useful for systemic action of protein-peptide drugs such as insulin, calcitonin, and met-enkaphalin and even for other non-peptide drugs such as cardiovascular and anti-asthmatic agents.

1.2 LIMITATIONS OF COLON SITE SPECIFICITY (4, 5, 6, 7):

1. As a site for drug delivery, the colon offers near neutral pH, reduced digestive enzymatic activity, a long transit time and increased responsiveness to absorption enhancers; however, the targeting of drugs to the colon is very complicated.

2. Due to its location at the distal portion of the alimentary canal, the colon is particularly difficult to access.

3. There is a wide range of pH values and different enzymes present throughout the GIT, through which the dosage form has to travel before reaching the target site, further complicate the reliability and delivery efficiency.

4. Successful delivery through this site also requires the drug to be in solution form before it arrives in the colon or, alternatively, it should dissolve in the luminal fluids of the colon, but this can be a limiting factor for poorly soluble drugs as the fluid content in the colon is much lower and it is more viscous than in the upper part of the GIT.

5. The stability of the drug is also a concern and must be taken into consideration while designing the delivery system. The drug could potentially bind in a nonspecific manner to dietary residues, intestinal secretions, mucus or fecal matter. The resident microflora could also affect colonic performance via metabolic degradation of the drug.

6. Lower surface area and relative ‘tightness’ of the tight junctions in the colon can also restrict drug transport across the mucosa and into the systemic circulation.
1.3 ANATOMY AND PHYSIOLOGY OF GIT

The GIT, also called the alimentary canal, is a muscular digestive tube that winds through the body. The GIT is a selective barrier between the environment and the systemic circulation, which functions to digest dietary food, to absorb nutrients, electrolytes and fluid, and to prevent the absorption of potentially harmful substances. The small intestine is the longest part of the GIT where most of the enzymatic digestion and virtually all absorption occur.

![Gastrointestinal Tract Diagram]

**Figure 1.1 Gastrointestinal Tract**

The large intestine is the last major subdivisions of the GIT. The digested materials that reach the large intestine contain few nutrients, but the residues remain here for 12-24 h. Major regions of the large intestine are the cecum, colon, rectum and anal canal (1, 8).

1.3.1 Structure of Colon

The colon forms the lower part of the GIT and extends from the ileo-caecal junction to the anus (4). The entire colon is about 5 feet (150cm) long which is divided into following groups.
Chapter 1: Introduction

Figure 1.2 Structure of Colon

- **Ascending Colon**
  20-25 cm long, located behind the peritoneum hepatic flexure- lies under right lobe of liver.

- **Cecum (Proximal Right Colon)**
  6x9cm pouch covered with peritoneum appendix a vermiform (wormlike) diverticulum’s located in the lower cecum.

- **Transverse Colon**
  Lies anterior in abdomen, attached to gastro colic ligament splenic flexure near tail of pancreas and spleen

- **Descending Colon**
  10-15cm long located behind the peritoneum. After it enters the true pelvis, it is known as a sigmoid colon.

- **Sigmoid Colon**
  This part describes an S-shaped curve in the pelvis that continues downwards to become the rectum.

- **Rectum**
  This is slightly dilated section of the colon about 13cm long. It leads from the sigmoid colon and terminates in the anal canal.

- **The anal canal**
  This is the short passage about 3.8cm long and leads from the rectum to the exterior.
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Both the ascending and descending colon are retroperitoneal; the transverse and sigmoid colon are not true to its name, the ascending colon side of the abdomen, reaches the inferior surface of the liver and turns abruptly to the left to form the right colic (colic) flexure. The colon continues across the abdomen to the left side as the transverse colon. It curves beneath the inferior end of the spleen on the left side as the colic (splenic) flexure and passes inferiorly to the level of the iliac crest as the descending colon. The sigmoid colon (sigma= s-shaped) begins near the left iliac crest, projects medially to the midline and terminates as the rectum at about the level of the third sacral vertebra. The rectum the last 20cm of the GIT, lies anterior to the sacrum and coccyx the terminal 2-3cm of the rectum is called the anal canal.

The major function of the colon is the consolidation of the intestinal contents into faeces by the absorption of water and electrolytes and to store the faeces until excretion. The absorptive capacity is very high; each day about 2000 ml of fluid enters the colon through the ileo-caecal valve from which more than 90 % of the fluid is absorbed (2, 7, and 8).

1.3.2 pH of the Colon

The pH of the GIT is subjected to both inter and intra subject variations. Table 1.1 gives an overview of the average pH of The GIT (2, 4).

<table>
<thead>
<tr>
<th>Location</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td>6.2 - 7.4</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>5.0 - 6.0</td>
</tr>
<tr>
<td>Stomach</td>
<td>Fasted condition: 1.5 - 2.0</td>
</tr>
<tr>
<td></td>
<td>Fed conditions: 3.0 - 5.0</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Jejunum: 5.0 - 6.5</td>
</tr>
<tr>
<td></td>
<td>Ileum: 6.0 - 7.5</td>
</tr>
<tr>
<td>Large intestine</td>
<td>Right colon: 6.4</td>
</tr>
<tr>
<td></td>
<td>Mild colon and left colon: 6.0 - 7.6</td>
</tr>
</tbody>
</table>
1.3.3 Gastro Intestine Transit

Gastric emptying of dosage forms is highly variable and depends primarily on whether the subject is fed or fasted and on the properties of the dosage form such as size and density. The arrival of an oral dosage form at the colon is determined by the rate of gastric emptying and the small intestinal transit time.

The transit times of small dosage forms in GIT are given in Table 1.2.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Transit Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>&lt;1 (fasting) &gt;3 (fed)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3 – 4</td>
</tr>
<tr>
<td>Large intestine</td>
<td>20 – 30</td>
</tr>
</tbody>
</table>

Diseases affecting colonic transit have important implications for drug delivery, diarrhea increases colonic transit and constipation decreases it. However, in most disease conditions, transit time appears to remain reasonably constant (2).

1.3.4 Colonic Bacteria

A large number of aerobic and anaerobic bacteria are present throughout the length of the GIT. The microbial flora of the colon is predominantly anaerobic bacteria such as bacteroides, bifidobacterium, eubacterium, peptococcus, and peptostreptococcus.

The bacterial count (CFU/ml) in different regions of the GIT is.

Stomach: $0 – 10^3$ CFU/ml
Jejunum: $0 – 10^5$ CFU/ml
Ileum: $10^3 – 10^7$ CFU/ml
Colon: $10^{11} – 10^{12}$ CFU/ml

These bacteria carry out variety of metabolic reactions like hydrolysis, decarboxylation, dealkylation, dehalogenation. The metabolic activity of the microflora can be modified
by various factors such as age, disease, intake of drugs, and fermentation of dietary residues. This may lead to inactivation of drugs and enhancement of the drug action and side effects (2, 7).

![Figure 1.3: Colonic Bacteria](image)

### 1.4 APPROACHES FOR TARGETING DRUGS TO THE COLON

By definition, an oral colonic delivery system should retard drug release in the stomach and small intestine but allow complete release in the colon. The system designed for the delivery of drug in the colon may be single or multiple unit dosage form which is based on the core being coated with one or more successive layers. Single unit colon targeted drug delivery systems may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of the potential benefits like increased bioavailability, reduced risk of local irritation and predictable gastric emptying (9). Multiparticulate approaches tried for colonic delivery includes formulations in the form of pellets, granules, micro particles and nanoparticles. The use of multiparticulate drug delivery systems in preference to single unit dosage forms for colon targeting showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these
systems are capable of passing through the GIT easily, leading to less inter and intra subject variability. Moreover, multiparticulate systems tend to be more uniformly dispersed in the GIT and also ensure more uniform drug absorption (10).

A variety of approaches have been used and systems have been developed for the purpose of achieving colonic targeting. These include (11):

a) Systems developed with pH sensitive polymers
b) Time-dependent formulations
c) Pressure dependent systems
d) Enzyme controlled release systems

1. pH-controlled drug release systems:
This approach is based on the pH-dependent release of the drug from the system. In this case the pH difference between the upper and terminal parts of GIT is exploited to effectively deliver drugs to the colon. The pH in the terminal ileum and colon (except ascending colon) is higher than in any other region of the GIT. Thus a dosage form that disintegrates preferentially at high pH levels has good potential for site-specific delivery into this region. These systems effectively resist drug release under acidic conditions of the stomach; a considerable amount of drug may be released in the small intestine before it reaches the colon. The pH-dependent systems 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) at the site of digestion 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. Some pH-dependent coating polymers are listed in Table 1.3 (10, 12).

The polymers used for colon targeting, however, 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 of slightly alkaline pH of the terminal ileum and preferably at the ileo-cecal junction. These processes distribute the drug throughout the large intestine and improve the potential of colon targeted delivery systems (12, 13, 14, and 15).
Table 1.3: List of pH dependent polymers

<table>
<thead>
<tr>
<th>pH dependent polymers</th>
<th>Threshold pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvinyl acetate phthalate (PVAP)</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose acetate phthalate (CAP)</td>
<td>6.0</td>
</tr>
<tr>
<td>Cellulose acetate trimellitate (CAT)</td>
<td>5.5</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose acetate succinate (HPMCAS)</td>
<td>≥5.5</td>
</tr>
<tr>
<td>LF Grade</td>
<td>≥6.0</td>
</tr>
<tr>
<td>MF Grade</td>
<td>≥6.8</td>
</tr>
<tr>
<td>HF Grade</td>
<td></td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose phthalate (HPMCP)</td>
<td>≥5.0</td>
</tr>
<tr>
<td>HP-50</td>
<td></td>
</tr>
<tr>
<td>HP-55 and HP-55S</td>
<td>≥5.5</td>
</tr>
<tr>
<td>Shellac</td>
<td>7.0</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type A</td>
<td>≥6.0</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type B</td>
<td>≥7.0</td>
</tr>
<tr>
<td>Methacrylic acid copolymer, Type C</td>
<td>≥5.5</td>
</tr>
<tr>
<td>Methacrylic acid copolymer dispersion</td>
<td>5.6</td>
</tr>
</tbody>
</table>

2. Time-controlled drug release systems:

Usually, time-dependent drug delivery systems are designed to deliver drugs after a lag of five to six hrs. This approach is based upon the theory that the lag time equates to the time taken for the dosage form to reach the colon. The lag time is dependent on size of dosage form and gastric motility associated with the pathological condition of the individual. Time-controlled systems are useful for synchronous delivery of a drug either at pre selected times such that patient receives the drug when needed or at a pre-selected site of the GIT. These systems are therefore particularly useful in the therapy of diseases, which depend on circadian rhythms. Time-controlled formulations for colonic delivery are also delayed-release formulations in which the delay in delivery of the drug is time-based. In these systems, the site of drug release is decided by the transit time of a
formulation in the GIT, which makes it challenging to develop a formulation in order to achieve a precise drug release in the colon. Ideally, formulations are designed such that the site of delivery (i.e. colon) is not affected by the individual differences in the gastric emptying time, pH of the stomach and small intestine or presence of anaerobic bacteria in the colon. On an average, an orally administered dosage form takes about 3 hrs to travel through the length of the small intestine to the beginning of the colon. Compared to gastric emptying rate, the small intestinal transit time is relatively consistent. In principle, time-controlled systems rely on this consistent small intestinal transit time. Drug release from this system typically occurs after a predetermined lag time, which corresponds to or exceeds the small intestinal transit time, to ensure drug delivery to the large intestine. In general, time-controlled formulations for colonic delivery include a pH-dependent (enteric coat) component because the transit of a formulation in the GIT is largely influenced by the gastric emptying time. Enteric coating is also used for preventing the rapid swelling and disintegration in upper GIT since other controlled-release components based on mechanism of swelling (gelling), osmosis or a combination of two are often included in the time-release formulations. An example of such a system is Pulsincap®. This capsule consists of a non-disintegrating body having an enteric coated cap. The enteric coated cap dissolves in the small intestine and a hydrogel plug swells to create a lag phase. This plug ejects on swelling and releases the drug from the capsule. Another example is Time Clock® which 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 coating slowly erodes away and the core is then available for dispersion (14, 15, 16, and 17).

Some commercial products based on this approach are: Pulsys® (Amoxicillin), Uniphyl® (Theophyllin) and Ritalinβ (Methyl phenidate) (10). Polymers suitable for Timed Release Systems are listed in Table 1.4 (10, 12).
3. *Pressure controlled drug release systems:*

GIT pressure is another mechanism that is utilized to initiate the release of the drug in the distal part of the gut. Viscosity of the luminal contents within the colon is greater than at other sites within the G.I tract due to the reabsorption of water from the large intestine. This change in viscosity leads to an increase in pressure resulting from the peristaltic forces. This pressure change can be used to trigger drug release. One example of such a system is push-pull OROS-CT system, which comprises of 5 push-pull units encapsulated within a hard gelatin capsule. Each push-pull unit is a bi-layered laminated structure containing an osmotic push layer and a drug layer, both surrounded by a semipermeable layer. An orifice is laser drilled into the semipermeable membrane to the drug layer. The outside surface of the semipermeable membrane is then coated by Eudragit® S-100 to delay the drug release from the device during its transit through the stomach. Upon arrival in the small intestine, the coating dissolves at a pH $\geq 7$. As a result, water enters the unit causing the osmotic push compartment to swell forcing the drug out of the orifice into the colon. The drug release kinetics is precisely controlled by the rate of influx of water through the semipermeable membrane (11, 12).

Another example of this system pressure-controlled colon delivery capsule (PCDC) made of ethyl cellulose has been developed to target the drugs to the colon. The PCDC is composed of drug, dispersed in a suppository base, and coated with hydrophobic polymer and ethyl cellulose. Once swallowed, the temperature of the body causes the suppository

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**Table 1.4: List of polymers suitable for Timed Release Systems**

- Hydroxy propyl methyl cellulose
- Hydroxyethyl cellulose
- Ethyl cellulose
- Microcrystalline Cellulose
- Hydroxy propyl methyl cellulose
base to melt and increase in volume and the system resembles a liquid-filled ethylcellulose balloon. The balloon is able to withstand the luminal pressure of the small intestine resulting from peristalsis, but will rupture when subject to the pressure of more intense contractions of the colon and contents of thicker viscosity (13).

4. **Enzyme controlled systems:**
Microflora activated delivery systems are considered to be preferable and promising since the abrupt increase of the bacteria population and associated enzymatic activities in ascending colon represents a non-continuous event independent of GIT transit time and pH (11).

- **Prodrug approach:**
Prodrugs are designed to undergo minimal absorption and hydrolysis in the upper GIT and undergo enzymatic hydrolysis in the colon, releasing the active drug moiety from the carrier. A prodrug is a pharmacologically inactive derivative of a parent molecule that requires spontaneous or enzymatic transformation within the body to release the active drug moiety. For targeting drugs to the colon, drug is to be protected from the hostile environments of the stomach and small intestine. This protection in the upper GIT is affected by conjugation with carrier moieties, forming prodrugs. These prodrugs undergo enzymatic cleavage in the colon and regenerate the drug. An example of such a prodrug, which is extensively used in Crohn’s disease and ulcerative colitis, is sulphasalazine. It consists of 5-aminosalicylic acid (5-ASA) linked via an azo bond to sulphapyridine (11, 12).

- **Polysaccharide Based Approach**
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. The bacterial amount has been estimated about 10 per gram in the colon and having around 400 species (anaerobic in nature). The important bacteria present in the colon such as *Bacteroides,*
Bifidobacterium, Eubacterium, Peptococcus, Lactobacillus, Clostridium secrete a wide range of reductive and hydrolytic enzymes such as β-glucuronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase. These enzymes are responsible for degradation of di-, tri- and polysaccharides. Earlier polymer cross linked with azo aromatic groups was used but due to potential carcinogenic activity, now days natural polysaccharides are used. Polysaccharides, the polymer of monosaccharide 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 polysaccharides and results in the degradation of the matrices. A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans, dextrin and locust bean gum have been investigated for their use in colon targeted drug delivery systems. Various enzymes that are involved in the degradation of some of these polymers are amylase, chitosanase, pectinase, inulinase, xylanase, dextranase, and galactomannanase (18, 19, and 20).

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 (20).

The polysaccharides naturally occurring in plant (e.g., pectin, guar gum, inulin), animal (e.g., chitosan, chondroitin sulphate), algal (e.g., alginates) or microbial (e.g., dextran) origin were studied for colon targeting.

Pectin is a non-starch linear polysaccharide that consists mainly of α-(1, 4) D-galacturonic acid and α-(1,2) L-rhamnose, found in the cell walls of plants. It is completely degraded by colonic bacteria but is not digested in the upper GIT. The
disadvantage of pectin is its solubility. To overcome this restriction the degree of its methoxylolation has been modified and also calcium pectinate has been prepared in order to make pectin resistant in the upper GIT. Combination of pectin and ethylcellulose was used to film coat paracetamol tablet cores. Drug release was depended on the nature and characteristics of the mixed film as well as the composition of the dissolution medium. 5-Aminosalicylic acid beads coated with pectin/ethylcellulose were prepared and evaluated for drug delivery to the colon. Simulated gastric fluid was found to influence drug release (Hydration and swelling characteristics of pectin), and also the ratio of pectin to ethylcellulose in the coat. Pectin has also been investigated in combination with chitosan and hydroxypropyl methylcellulose (HPMC).

Pectin/chitosan and HPMC mixtures have been investigated as a film coating system for colonic delivery, forming in situ polyelectrolyte complex between pectin and chitosan. *In vitro* and *in vivo* investigations were carried out using such systems. In vitro dissolution of the tablets using pectinolytic enzyme showed that the release rate was faster than in the absence of this enzyme. It has also been found that the tablets coated with pectin: chitosan: HPMC were able to pass the stomach and small intestine intact, but once the tablets arrive into the colon started to disintegrate when administered to human volunteers. Eudragit S-coated pectin microspheres of 5- fluorouracil have been prepared and evaluated for colon targeting in order to reduce side effects of the drug caused by its absorption from the upper part of the GIT. As expected, drug release could be suppressed in simulated gastric fluid and triggered at pH 7.4. *In vitro* drug release study in the presence of rat cecal content have shown that there are no/slightly difference between the release profile in the presence and absence of cecal content (20, 21 and 22).

*Chitosan* is the second most abundant polysaccharides in nature after cellulose, obtained by the alkaline N-deacetylation of chitin. Chitosan molecule is a copolymer of N-acetyl-D-glucosamine and D-glucosamine. Chitosan was used in oral drug formulations to provide colonic drug delivery. Chitosan is also considered as a promising candidate for colon targeting because of its favorable biological properties (e.g., non-toxicity, biocompatibility and biodegradability). Chitosan is degraded by the colonic microflora,
and it is not digested in the upper part of the GIT by human digestive enzymes. Drug delivery systems utilizing chitosan is discussed by various researchers. Insulin and 5-aminosalicylic acid have been administered to rats in enteric-coated chitosan capsules. Recently, a tablet formulation was developed using chitosan, guar gum as carriers in the matrix-tablet, and then was coated firstly with inulin as inner coat, and secondly with shellac as outer coat. The investigated tablet has controlled the drug release in gastric and intestinal fluids, however, drug release was found to be enhanced in the presence of rat cecal contents. Chitosan-Ca-alginate microparticules have been prepared and characterized to deliver 5-aminosalicylic acid to the colon after oral administration. Chitosan-prednisolon conjugate microspheres were coated with eudragit L 100 and evaluated in vitro at different pH levels. Microspheres coated with eudragit are able to protect drug in simulated gastric fluid but once the pH increased to 6.8 the release rate of the microspheres increased significantly (23, 24, and 25).

*Guar gum* derived from the seeds of *Cyamopsis tetragonolobus* is a naturally occuring galactomannan polysaccharide. It is made up of a linear chain of β-D-mannopyranose joined by β-(1-4) linkage with α-D-galactopyranosyl units attached by 1, 6-links in the ratio of 1:2. Guar gum contains about 80% galactomannan, 12% water, 5% protein, 2% acid insoluble ash, 0.7% ash and 0.7% fat. Guar gum hydrates and swells in cold water forming viscous colloidal dispersions or sols. This gelling retards the drug release from the tablets. Guar gum is being used to deliver drug to the colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine. Guar gum based matrix tablets of dexamethasone and other anti-inflammatory agents have shown very encouraging results as colon-carriers. Matrix tablets of dexamethasone and budesonide were prepared using 60.5% w/w of guar gum in the tablet. The study showed negligible drug release in simulated gastric and intestinal fluid whereas in simulated colonic fluid significant increase in drug release was observed. The study demonstrated that the galactomannananase (> 0.1%) accelerated dissolution of dexamethasone and budesonide from guar gum matrix tablet. The extent of drug dissolution depended on concentration of galactomannanase (20, 24, and 26).
Starch is a polysaccharide which occurs as microscopic granules in the tissues of many plants species, is degraded by many bacterial species (e.g., bacteroides, bifidobacteria). Starch is composed of two polysaccharides: amylose and amylopectin. Amylose is an essentially linear α-glucan containing α-(1, 4) bonds. Amylopectine has a much higher molecular weight than amylose and is much more heavily branched, with about 95% α-(1, 4) and 5% α-(1, 6) bonds. The amount of amylose usually present in starch is between 20% and 35%. Breeders have developed starches which contain amylose between 50% and 80%. Resistant starch to digestive enzymes (e.g., pancreatin enzymes within the small intestine) can be made by the formation of an amorphous structure (amorphous amylose) though can be degraded by colonic bacteria. However, not all forms of amylose are resistant to digestion in the upper GIT. For this reason, glassy amylose was chosen to provide colonic drug delivery, besides, only retrograded amylose resists upper GIT digestion by pancreatic enzymes and also due to its microstructure, amylose has been used in coatings of colon-specific formulations. A disadvantage of amylose in film form is its swelling in aqueous media and subsequent accelerated drug release. Pure amylose films take up considerable amounts of water upon exposure to aqueous media. They become very permeable and the drug is already released in the upper GIT before the distal GIT is reached. A formulation which provides improved controlled targeted release of an oral administration of prednisolone sodium metasulphobenzoate to the colon has been developed in order to decrease systemic absorption and consequently low risk of systemic adverse events of corticosteroids. The formulation comprises prednisolone sodium metasulphobenzoate surrounded by glassy amylose: ethylcellulose (ratio from 1:3.5 to 1:4.5) plasticized with dibutyl sebacate. The formulation has shown that the drug delivery starts by the arrival of the dosage form in the colon. An ethylcellulose/glassy amylose surrounded formulation is now available as COLAL®, which has been used to coat pellets containing the corticosteroid prednisolone sodium metasulphobenzoate (COLALPRED®; Alizyme Therapeutics Ltd, Cambridge, UK). This product has achieved successful phase II clinical trial results and is now in phase III clinical trials for the treatment of moderate to severe ulcerative colitis. Mixed amylose /eudragit coating dispersion has also been used to delay drug release and target the colon (20, 24, and 26).
**Chondroitin sulphotate** is a soluble mucopolysaccharide that is used as a substrate by the bacteroides (e.g., *Bacteroides ovatus*) of the large intestine. Chondroitin sulphate could be used as a carrier for colon targeted delivery of bioactive agents. In contrast to natural chondroitin sulphate, which is readily water-soluble and not able to prevent drug release in the upper GIT, cross-linked chondroitin sulphate would be less hydrophilic and thus would provide a better drug controlling in the stomach and small intestine. Cross-linked chondroitin sulphate in matrix formulations with indomethacin as a drug carrier was investigated to control drug release in the colon. *In vitro* indomethacin release upon exposure to phosphate buffer with and without rat cecal content has shown that the faster drug release depended on the biodegradation action of bacterial enzymes (19, 24).

**Inulin** is a naturally accruing glucofructan found in many plants. It consists of β-(1-2) linked D-fructose molecules having a glucosyl unit at the reducing end. Inulin is not significantly hydrolyzed by digestive enzymes in the upper GIT, however, colonic bacteria and more specifically bifidobacteria can degrade this polysaccharide. It can serve as a biodegradable compound with eudragit RS if an inulin-type with a high degree of polymerization is used to lower its water solubility. Mixed films of inulin and eudragit RS withstand gastric and intestinal fluids which indicate that this coating system could also serve as coating materials for colon targeting. The bacterial degradation has been shown to depend on the hydrophilicity of the plasticizer. However, eudragit RS and RL in combination with inulin made free films have been shown more swelling and permeation of drug in colonic medium rather than in gastric and intestinal fluids (20, 24).

**Alginites** are natural hydrophilic polysaccharide derived from seaweed. They are linear polymers consisting of (1-4)-β- D mannuronic acid and α-L glucuronic acid residues. The gelation of alginites can be induced by adding Ca++ ions because alginites do not gel since they have poly (L-glucuronic acids) which are rigid. 5-aminosalicylic acid has been sprayed on calcium alginate cores for the use in targeted drug delivery systems. In this spray, beads were coated with different percentages of enteric coating polymer and/or sustained release polymer (eudragit L 30D, aquacoat). Alginate beads were also coated
with dextran acetate. Drug release was significantly faster in the presence of dextranase than in the absence of this enzyme (20, 27).

**Dextrans** are a class of polysaccharides with a linear polymer backbone with mainly 1,6-\(\alpha\)-D-glucopyranoside linkages with side chains of additional \(\alpha\)-(1,4) and \(\alpha\)-(1,3) bonds. Dextran has been found to be degraded in human feaces due to bacterial action. Various drug-dextran prodrugs in which the drug molecule is linked to the polar dextran macromolecule remain intact and unabsorbed from the stomach and small intestine but when the prodrug enters into the colonic environment is degraded by dextranases. Dextran and 5- aminosalicylic acid conjugates were synthesized and evaluated for drug delivery to the colon (20, 24).

**Cyclodextrins** are cyclic oligosaccharides. They consist of 6–8 glucose units linked through \(\alpha\)-1, 4- glucosidic bonds. Cyclodextrins are neither hydrolyzed nor absorbed from the stomach and small intestine. However, in the colon the vast microflora present breaks these into small saccharides and thus is absorbed in the large intestine. This property of being able to be degraded by colonic bacteria especially *Bacteroides* led to its use as a colon targeting carrier. Ester conjugates of biphenyl acetic acid with \(\beta\)-cyclodextrin released the drug preferentially when incubated with rat cecal contents and almost no release was observed on incubation with contents of stomach and small intestine (20, 24).

**Locust bean gums** were found to be soluble in water. Cross-linked gums however led to water-insoluble film forming product showing degradation in colonic microflora. However, dissolution study performed on theophylline tablets coated with cross-linked locust bean gum showed the mechanical instability of these coatings in the dissolution media, thereby suggesting the non-suitability of such films as colon carriers (24).
II. MICROSPHERES AS A DRUG CARRIER

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200µm.

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. One such approach is using microspheres as carriers for drugs (28).

Advantages of the technique are:

1. Less gastric irritation.
2. Dose dumping is minimum
3. High therapeutic index is obtained.
4. The microsphere based carrier can be injected in the body in suitable vehicle using hypodermic needle.
5. Helpful in passive targeting of drug liver and spleen.

Disadvantages are:

1. Maintenance of particle size is difficult.

1.1 POLYMERS USED FOR THE FABRICATION OF MICROSPHERES

Polymers used for the fabrication of microspheres, are classified into two types (29):

1. **Synthetic Polymers** are divided into two types.
   a. Non-biodegradable polymers
      E.g. Poly methyl methacrylate (PMMA)
      Acrolein
      Glycidyl methacrylate
   b. Biodegradable polymers
      E.g. Lactides, Glycolides & their co polymers
      Poly alkyl cyano acrylates
      Poly anhydrides
2. **Natural Polymers** obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.

- **Proteins**: Albumin, Gelatin, and Collagen
- **Carbohydrates**: Agarose, Carrageenan, Chitosan, Starch, Pectin
- **Chemically modified carbohydrates**: Poly dextran, Poly starch

1.2 METHODS OF PREPARATION

1. **Single Emulsion Technique**

   The micro particulate carriers of natural polymers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. Next cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, di-acid chloride etc.

2. **Double Emulsion Technique**

   Double emulsion method of microsphere preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited to water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is subjected then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like leutinizing hormone releasing hormone (LH-RH) agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/extraction (29, 30).
3. Polymerization Techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

- Normal polymerization
- Interfacial polymerization

Both are carried out in liquid phase.

- Normal Polymerization

It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers (30).

- Interfacial Polymerization

It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase (28).

4. Spray Drying and Spray Congealing

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization
leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100µm. Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying (30).

5. **Solvent Evaporation**

Solvent evaporation method is used for the preparation of microparticles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for the microspheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer (29).

**III. REFERENCES**