CHAPTER-1

GENERAL INTRODUCTION
Cancer

A cell is the smallest living entity in the body and is often known as the structural and functional unit of all living organisms. Normal body cells grow, divide and die in an orderly fashion [1]. All the functions of a cell including rate of cell growth, division, differentiation and death are regulated by a specified set of genes, which act as triggers. A loss of function of these genes, sometimes lead to a state of uncontrolled cell growth without any differentiation; termed as “Neoplasia or Cancer”.

Cancer cells divide and produce new cells in an uncontrolled way that can spread throughout the body and cause damage to essential organs. When cancer spreads to other parts of the body, this is called metastasis. Metastasis can occur when cancer cells enter the bloodstream or lymph system. These systems circulate all over the body and allow the cells to travel and deposit at other parts of body [2].

Overview of Cancer

Researchers divide the causes of cancer into two groups: first with an environmental cause and second with a hereditary genetic cause. Cancer is primarily an environmental disease, though genetics influence the risk of some cancers. Common environmental factors leading to cancer include: tobacco, diet and obesity, infections, radiation, lack of physical activity, and environmental pollutants. These environmental factors cause or enhance abnormalities in the genetic material of cells [3]

Estimated Cases and Deaths

According to the new edition of the World Cancer Report from the International Agency for Research on Cancer, due to rapid increase in cancer cases, cancer will soon replace heart disease as the leading cause of deaths worldwide. Report added that low- and middle-income countries will experience the impact of higher cancer incidence and death rates than industrialized countries. Cancer will become No. 1 cause of death worldwide by 2014. Report projected that 2.4 million people will be diagnosed with some form of cancer this year and 7.6 million people will die. Report said: "The global cancer burden doubled in the last 30 years of the 20th century, and it is estimated that this will double again between 2000 and 2020 and nearly triple by 2030."
This list of common cancer types and their estimated number are given in table 1A.

**Table 1A: Estimated number of new cases and deaths for each common cancer type**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>70,980</td>
<td>14,330</td>
</tr>
<tr>
<td>Breast (Female - Male)</td>
<td>192,370 - 1,910</td>
<td>40,170 - 440</td>
</tr>
<tr>
<td>Colon and Rectal (Combined)</td>
<td>146,970</td>
<td>49,920</td>
</tr>
<tr>
<td>Endometrial</td>
<td>42,160</td>
<td>7,780</td>
</tr>
<tr>
<td>Kidney (Renal Cell) Cancer</td>
<td>49,096</td>
<td>11,033</td>
</tr>
<tr>
<td>Leukemia (All)</td>
<td>44,790</td>
<td>21,870</td>
</tr>
<tr>
<td>Lung (Including Bronchus)</td>
<td>219,440</td>
<td>159,390</td>
</tr>
<tr>
<td>Melanoma</td>
<td>68,720</td>
<td>8,650</td>
</tr>
<tr>
<td>Non-Hodgkin Lymphoma</td>
<td>65,980</td>
<td>19,500</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>42,470</td>
<td>35,240</td>
</tr>
<tr>
<td>Prostate</td>
<td>192,280</td>
<td>27,360</td>
</tr>
<tr>
<td>Skin (Nonmelanoma)</td>
<td>&gt;1,000,000</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td>Thyroid</td>
<td>37,200</td>
<td>1,630</td>
</tr>
</tbody>
</table>

**Cancer cell biology**

Cancer is a diverse class of diseases, which differ widely in their causes. Research into the pathogenesis of the cancer can be divided into three broad area of focus. The first area of the research focus on the agents and the events which cause or facilitate genetic changes in the cells destined to become cancer. Second, it is important to overcome the precise nature of the genetic damage, and the genes which are affected by it. The third focus is on the consequences of those genetic changes on the biology of the cells, both in the generating the defining properties of the cancer cell, and in facilitating additional genetic events, leading to the further progression of the cancer.
Genetic abnormality found in the cancer typically affects two general classes of genes:

- **Cancer- promoting Oncogenes** - Oncogenes promote cell growth through a variety of ways. Oncogenes often produce mutagens, or are involved in transcription of DNA in protein synthesis, which creates the proteins and enzymes responsible for producing the products and bio-chemicals used by the cells.

- **Tumor suppressor genes** - The functions of such genes is to arrest the progression of the cell cycle in order to carry out DNA repair, preventing mutations from being passed on to daughter cells. The p53 protein, one of the most important studied tumor suppressor genes, is transcription factor activated by many cellular stressors including hypoxia and ultraviolet radiation damage.

Fig 1A: Cancer cell biology [5]
Classification of Cancers

There are five broad groups that are used to classify cancer

1. **Carcinomas** - Cancer that begins in the skin or in tissues that line or cover internal organs.
2. **Sarcomas** - Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
3. **Lymphomas** - Cancers that begin in the cells of the immune system.
4. **Leukaemia’s** - Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
5. **Central nervous system cancers** - Cancers that begin in the tissues of the brain and spinal cord.

**Signs and symptoms [7]**

- Local symptoms: unusual lumps or swelling (tumor), haemorrhage (bleeding), pain and/or ulceration.
- Symptoms of metastasis (spreading): enlarged lymph nodes, cough and haemoptysis, hepatomegaly (enlarged liver), bone pain, fracture of affected bones

Fig 1B: cancer cell proliferation and metastasis to other organs [6]
and neurological symptoms. Although advanced cancer may cause pain, it is often not the first symptom.

- Systemic symptoms: weight loss, poor appetite, fatigue and cachexia (wasting), excessive sweating (night sweats), anaemia and specific paraneoplastic phenomena, i.e. specific conditions that are due to an active cancer, such as thrombosis or hormonal changes.

**Cancer Treatments [8]**

The treatment given for cancer is highly variable and dependent on a number of factors including the type, location and amount of disease and the health status of the patient. The treatments are designed to either directly kill/remove the cancer cells or to lead to their eventual death by depriving them of signals needed for cell division. Other treatments work by stimulating the body's own defences. Cancer can be treated by surgery, Chemotherapy [9], Radiation therapy, Immunotherapy [10], Monoclonal antibody therapy [11], Hormone therapy [12], Photodynamic therapy [13], Cancer vaccine [14], Targeted therapy [15] and other methods.

**Colon cancer**

No matter where a cancer may spread, it is always named for the place where it started. For example, breast cancer that has spread to the liver is still called breast cancer, not liver cancer. Colon cancer is cancer that starts in the colon. Colon cancer starts in the innermost layer and can grow through some or all of the other layers.

**Anatomy of colon [16]**

The locations of the parts of the colon are either in the abdominal cavity or behind it in the retro peritoneum. The colon in those areas is fixed in location. The Haustra of the colon are the small pouches caused by sacculation, which give the colon its segmented appearance. The taenia coli run the length of the large intestine. As the taenia coli is shorter than the intestine, the colon becomes sacculated between the taenia, forming the Haustra.
In mammals, the colon consists of four sections: the ascending colon, the transverse colon, the descending colon, and the sigmoid colon (the proximal colon usually refers to the ascending colon and transverse colon). The colon, cecum, and rectum make up the large intestine.

**Ascending colon**
The ascending colon, on the right side of the abdomen, is about 25 cm long in humans [18]. It is the part of the colon from the cecum to the hepatic flexure (the turn of the colon by the liver). It is secondarily retroperitoneal in most humans. In ruminant grazing animals, the cecum empties into the spiral colon.

Interiorly it is related to the coils of small intestine, the right edge of the greater omentum, and the anterior abdominal wall. Posterior, it is related to the iliacus, the iliolumbar ligament, the quadratus lumborum, the transverse abdominis, the diaphragm at
the tip of the last rib; the lateral cutaneous, ilioinguinal, and iliohypogastric nerves; the iliac branches of the iliolumbar vessels, the fourth lumbar artery, and the right kidney.

Arterial supply of the ascending colon comes from the ileocolic artery and right colic artery, both branches of the SMA. While the ileocolic artery is almost always present, the right colic may be absent in 5–15% of individuals.

**Transverse colon**
The transverse colon is the part of the colon from the hepatic flexure to the splenic flexure (the turn of the colon by the spleen). The transverse colon hangs off the stomach, attached to it by a wide band of tissue called the greater omentum. On the posterior side, the transverse colon is connected to the posterior abdominal wall by a mesentery known as the transverse mesocolon.

The transverse colon is encased in peritoneum and is therefore, mobile (unlike the parts of the colon immediately before and after it). Cancers form more frequently further along the large intestine as the contents become more solid (water is removed) in order to form feces.

**Descending colon**
The descending colon is the part of the colon from the splenic flexure to the beginning of the sigmoid colon. The function of the descending colon in the digestive system is to store food that will be emptied into the rectum. It is retroperitoneal in two-thirds of humans. In the other third, it has a (usually short) mesentery. The arterial supply comes via the left colic artery.

**Sigmoid colon**
The sigmoid colon is the part of the large intestine after the descending colon and before the rectum. The name *sigmoid* means S-shaped (see sigmoid). The walls of the sigmoid colon are muscular, and contract to increase the pressure inside the colon, causing the stool to move into the rectum.

**Redundant colon**
One variation on the normal anatomy of the colon occurs when extra loops form, resulting in a longer than normal organ. This condition, referred to as redundant colon,
typically has no direct major health consequences, though rarely volvulus occurs resulting in obstruction and requiring immediate medical attention [19]. A significant indirect health consequence is that use of a standard adult colonoscopy is difficult and in some cases impossible, when a redundant colon is present, though specialized variants on the instrument (including the paediatric variant) are useful in overcoming this problem.

An overview of the average pH in G.I.T. [Table-2] and transit time in G.I.T. [Table-3] is tabled below with their specification data:

**Table 1B: Average pH in G.I.T.**

<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>Esophagus</td>
<td>5.0-6.0</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.5-2.0 (fasting)</td>
</tr>
<tr>
<td></td>
<td>3.0-5.0 (fed)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>5.0-6.5 (jejenum)</td>
</tr>
<tr>
<td></td>
<td>6.0-7.5 (ileum)</td>
</tr>
<tr>
<td>Large intestine</td>
<td>6.4 (right colon)</td>
</tr>
<tr>
<td></td>
<td>6.0-7.6 (mid colon &amp; left colon)</td>
</tr>
</tbody>
</table>

**Table 1C: Transit time in G.I.T.**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Transit time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>&lt;1 (fasting) &gt;3</td>
</tr>
<tr>
<td></td>
<td>(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>

**Signs and symptoms of colorectal cancer [20]**

The symptoms of colorectal cancer depend on the location of tumor in the bowel, and whether it has spread elsewhere in the body (metastasis). Most of the symptoms may occur in other diseases as well, and hence none of the symptoms mentioned here is diagnostic of colorectal cancer. Symptoms and signs are divided into local, constitutional (affecting the whole body) and metastatic (caused by spread to other organs).

1. **Local**

Local symptoms are more likely if the tumor is located closer to the anus. There may be a change in bowel habit (new-onset constipation or diarrhoea in the absence of another cause), and a feeling of incomplete defecation (rectal tenesmus). Lower gastrointestinal bleeding, including the passage of bright red blood in the stool, may indicate colorectal cancer, as may the increased presence of mucus. Melena, black stool with a tarry
appearance, normally occurs in upper gastrointestinal bleeding (such as from a duodenal ulcer), but is sometimes encountered in colorectal cancer when the disease is located in the beginning of the large bowel.

A tumor that is large enough to fill the entire lumen of the bowel may cause bowel obstruction. This situation is characterized by constipation, abdominal pain, abdominal distension and vomiting. This occasionally leads to the obstructed and distended bowel perforating and causing peritonitis. A large left colonic tumor may compress the left urethra and cause hydronephrosis.

Certain local effects of colorectal cancer occur when the disease has become more advanced. A large tumor is more likely to be noticed on feeling the abdomen, and it may be noticed by a doctor on physical examination. The disease may invade other organs, and may cause blood or air in the urine (invasion of the bladder) or vaginal discharge (invasion of the female reproductive tract).

2. Constitutional

If a tumor has caused chronic occult bleeding, iron deficiency anaemia may occur; this may be experienced as fatigue, palpitations and noticed as pallor (pale appearance of the skin). Colorectal cancer may also lead to weight loss, generally due to a decreased appetite. More unusual constitutional symptoms are an unexplained fever and one of several paraneoplastic syndromes. The most common paraneoplastic syndrome is thrombosis, usually deep vein thrombosis.

Abnormal growths in the colon

In most people, colorectal cancers develop slowly over several years. Before a cancer develops, a growth of tissue or tumour usually begins as a non-cancerous polyp on the inner lining of the colon. A tumor is abnormal tissue and can be benign (not cancer) or malignant (cancer). A polyp is a benign, non-cancerous tumor. Some polyps can change into cancer but not all do. The chance of changing into a cancer depends upon the kind of polyp.

Adenomatous polyps (adenomas): are polyps that can change into cancer. Because of this, adenomas are called a pre-cancerous condition.
Hyperplastic polyps and inflammatory polyps: In general, are not pre-cancerous. But some of them can become pre-cancerous or might be a sign of having a greater risk of developing adenomas and cancer, particularly when these polyps grow in the ascending colon.

Fig 1D: Photograph showing polyps and cancer [40]

Types of cancers in the colon

Several types of cancer can start in the colon.

- **Adenocarcinomas:** More than 95% of colorectal cancers are a type of cancer known as adenocarcinomas. These cancers start in cells that form glands which make mucus to lubricate the inside of the colon and rectum.

- **Carcinoid tumors:** These tumors start from specialized hormone-producing cells in the intestine like Gastrin, Secretin etc.

- **Gastrointestinal stromal tumors (GISTs):** These tumors start from specialized cells in the wall of the colon called the interstitial cells. Some are benign (noncancerous); others are malignant (cancerous). These tumors can be found anywhere in the digestive tract, but they are unusual in the colon.

- **Lymphomas:** These are cancers of immune system cells that typically start in lymph nodes, but they may also start in the colon, rectum, or other organs.

- **Sarcomas:** These tumors can start in blood vessels as well as in muscle and connective tissue in the wall of the colon and rectum. Sarcomas of the colon or rectum are rare.
**Main causes of colon cancer**

Some genes contain instructions for controlling our cells growth, division, and death. Certain genes that speed up cell division or help cells to live longer are called *Oncogenes*. Others that slow down cell division, or cause cells to die at the right time, are called *tumor suppressor genes*. Cancers can be caused by DNA mutations (defects) that turn on Oncogenes or turn off tumor suppressor genes. Some DNA mutations may be passed from generation to generation and are found in all cells in the body.

**Inherited gene mutations**

A small portion of colorectal cancers are known to be caused by inherited gene mutations. For example, inherited changes in a gene called APC are responsible for familial adenomatous polyposis (FAP) and Gardner syndrome. The APC gene is a tumor suppressor gene - it normally helps to keep cell growth in check. In people who have inherited changes in the APC gene, this "brake" on cell growth is turned off, causing hundreds of polyps to form in the colon. Hereditary nonpolyposis colon cancer (HNPCC), also known as Lynch syndrome, is caused by changes in genes that normally help a cell repair faulty DNA. The rare Peutz-Jeghers syndrome is caused by inherited changes in the STK11 gene. This seems to be a tumor suppressor gene; although it’s exact function is not clear.

**Acquired gene mutations**

In most cases of colorectal cancer, the DNA mutations that lead to cancer are acquired during a person's life rather than having been inherited. In many cases, the first mutation occurs in the APC gene. This leads to an increased growth of colorectal cells because of the loss of this "brake" on cell growth.

**Cancer chemotherapy and its problem**

Chemotherapy affects cell division, tumors with high *growth fractions* (such as acute myelogenous leukaemia and the aggressive lymphomas, including Hodgkin's disease) are more sensitive to chemotherapy, as a larger proportion of the targeted cells are undergoing cell division at any time. Malignancies with slower growth rates, such as indolent lymphomas, tend to respond to chemotherapy much more modestly.
Chemotherapeutic techniques have a range of side effects that depend on the type of medications used are listed below-

- Depression of the immune system, which can result in potentially fatal infections. Although patients are encouraged to wash their hands, avoid sick people, and to take other infection-reducing steps, about 85% of infections are due to naturally occurring microorganisms in the patient's own gastrointestinal tract (including oral cavity) and skin.[20]

- Fatigue. The treatment can be physically exhausting for the patient, who might already be very tired from cancer-related fatigue. It may produce mild to severe anaemia.

- Tendency to bleed easily. Medications that kill rapidly dividing cells or blood cells are likely to reduce the number of platelets in the blood, which can result in bruises and bleeding. Extremely low platelet counts may be temporarily boosted through platelet transfusions.

- Gastrointestinal distress. Nausea and vomiting are common side effects of chemotherapeutic medications that kill fast-dividing cells. This can also produce diarrhoea or constipation. Malnutrition and dehydration can result when the patient doesn't eat or drink enough, or when the patient vomits frequently, because of gastrointestinal damage.

- Hair loss. Some medications that kill rapidly dividing cells cause dramatic hair loss; other medications may cause hair to thin. These are temporary effects: hair usually starts growing back a few weeks after the last treatment, sometimes with a tendency to curl that may be called a "chemo perm".

Novel drug delivery systems and marketed formulations

Today, the design and development of novel per oral delivery systems for peptides and proteins are the main goal of many pharmaceutical researchers. The low oral bioavailability of these peptide and proteins due to their low permeation across the intestinal epithelium, the harsh environment of the gastric pH, their rapid degradation by the proteolytic enzymes and their rapid clearance due to the first pass effect are the major drawbacks of developing a successful delivery system. However, the delivery of the hydrophilic peptide drug is not difficult to achieve but to enable its absorption in the intestinal tract is the crucial part. Hence, the delivery system has not only to overcome
the harsh pH of the stomach and the enzymatic degradation of the proteins in the GI tract, but also to increase the permeation of these molecules across the GI epithelium either by opening the tight junctions and increasing the paracellular transport or by increasing the endocytotic passage of the molecules through intracellular transport. In order to achieve this, the delivery system must be able to attach to a specific site in the GI tract long enough for the drug to permeate across the epithelium before the delivery system is being detached by the peristaltic movements of the gut [21]. A number of peroral delivery systems have been designed using liposomes, beads, adhesive drug delivery systems, superporous hydrogels etc.

**Liposome**

Long circulating macromolecular carriers such as liposomes can exploit the enhanced permeability and retention effect for the protein drugs. Liposomes are vesicles consisting of one to several, chemically active lipid bilayers. Drug molecules can be encapsulated and solubilised within these bilayers. Different types of phospholipids such as phosphotidyl choline or phosphotidyl inositol may be used in liposomal carriers. Liposomes are prepared by sonication, reverse phase evaporation or film formation [22]. Among different types of liposomes, dehydrated-rehydrated vesicles are most commonly used in protein drug delivery due to the ease of preparation and low amount of stress applied to the proteins [22]. The liposomes can be easily decorated with targeting moieties, e.g., antibodies, hence delivering the protein drugs to their specific target site [23, 24]. The liposomal composition, encapsulation efficiency, the rate of drug release from lipid bilayers, size and the surface charge are all important factors in successful liposomal drug delivery [25]. Stefanov et al. have used liposomes prepared from phosphotidylcholine (PC) and cholesterol (CH) for oral insulin delivery. They have reported a significant reduction in blood glucose levels in diabetic rats. Further investigations with liposomes containing insulin in rats and dogs showed reduction in blood glucose levels [26, 27]. Although liposomes with their organized structures have some advantages as drug delivery systems, the extensive leakage of water-soluble drugs entrapped in liposomes during the GIT passage, the low drug entrapment, the heterogeneity of the vesicle size, the poor reproducibility and instability of formulations are some of the disadvantages of using liposome as peptide/protein drug delivery system.
**Micro tablets**

Micro tablets with diameters of 0.5-3 mm containing permeation enhancers and/or enzyme inhibitors have been designed and investigated for the peroral delivery of protein and peptide drugs. The permeation enhancers must be released rapidly from the dosage form and prior to the release of the peptide over a wide area across the epithelium. In order for the peptide to pass through the epithelium, the site of opening of the paracellular pathway must coincide with the site where the peptide is released from the dosage form [28]. Hence, multiple unit dosage forms (MUDFs) were designed to control the release of the drug [29, 30]. The mini tablets can be then filled in gelatin capsules and enteric coated to be protected from the acidic condition of the stomach. Microtablets are easy to manufacture, can be used in defined sizes and strengths and show low variability within a batch [30]. In a study by Van der Merve et al. minitablets containing TMC Permeation enhancer and Desmopressin (1-(3-mercaptopropionic acid)-8-D-arginine Vasopressin monoacetate (DDAVP) were designed. The release of both DDAVP and TMC from different formulations of multi tablets was investigated [31]. The results suggested that suitable DDAVP release was obtained only from formulations that were too big to be fit inside the largest available gelatin capsule rendering them unsuitable for *in vivo* usage.

**Microspheres**

Spherical microspheres, prepared by complexation between oppositely charged macromolecules such as chitosan and negatively charged molecules such as tripolyphosphate (TPP) or alginates have received a lot of attention as drug delivery vehicles for protein drug delivery purposes [32, 33]. These microspheres can protect the drugs from the hostile environment of the GI tract, improve drug absorption via the paracellular route and control the drug release at a specific site [34-36]. Lueßen et al. and Kotzé et al. have applied drug containing chitosan microspheres on Caco-2 cell monolayers and showed a strong increase in the transport of buserelin, insulin and vasopressin derivative [36, 37]. A number of investigations were done by Shu et al. and Mutara et al. for controlled release drug delivery [38, 39]. They showed that variables such as drug concentration, type and concentration of chitosan, the pH of TPP solution, volume of the internal and external phases, gelation time as well as drying conditions can all determine the fate of drug release from chitosan beads. Avadi et al. have used enteric
coated capsules containing Brilliant Blue chitosan beads as model hydrophilic drug for colon drug delivery [40]. The $Y$-scintigraphy images have demonstrated that Eudragit S coated capsules containing Brilliant Blue loaded–chitosan beads is suitable for colon drug delivery. It can be thus concluded that the non toxic chitosan microspheres and beads can increase the bioavailability of the peptide and protein drugs by protecting them from degradation, when they are able to mucoadhesivity attach to a specific site on the intestinal tract and to increase drug permeation by opening the tight junctions via the paracellular pathway.

**Mucoadhesive drug delivery systems**
Mucoadhesion is the attachment of any type of polymer to the mucus layer via strong interaction between the functional groups of the polymer and those of the mucosa lining of the tissue. The mucoadhesive bonding is attained mostly by physical, chemical and more importantly through H-bonding. Hence, the presence of hydroxyl, carboxyl and H-bond forming functional groups strongly contributes to the strength of mucoadhesion [41, 42]. The formation process of mucoadhesive bonds include 1) wetting and swelling of the polymers, 2) interpenetration of the mucoadhesion polymer chains and entanglement of the polymer and mucin chains, 3) interfacial interaction of functional groups, 4) formation of weak chemical bonds. The use of mucoadhesive drug delivery systems results in a controlled drug release and attachment at a specific site of the body. Increasing the residence time of the drug delivery systems at the site of absorption in the body may result in prolonging their action. As the GI tract is covered by a mucus layer, the mucoadhesive drug delivery system must be able to attach to a specific site in order to be beneficial. Acrylic acid based polymers have been used extensively for mucoadhesive applications. Their strong bond strength in contact with tissues allows localization of the drug at the site of absorption, increasing residence time at the absorbing tissue and increasing drug bioavailability. Their responsive behaviour to different pH allows the drug to be released at the desired site of the GI tract [43]. In order to increase mucus interpenetration, adhesion promoters such as polyethylene glycol (PEG) may be employed, which are not mucoadhesive but contribute to the adhesion process. Moreover, these tethered promoters may be grafted onto polymeric surfaces such that at the one end they are covalently attached to the polymer surface and the other end is free. These
grafted chains are able to diffuse into the mucus layer and enhance the mucoadhesiveness of the system [44].

Peppas et al. have done extensive studies on the design and the effect of network morphology of polyethylene glycol (PEG) tethered copolymers as novel mucoadhesive drug delivery systems [45, 46]. They have suggested that the performance of the copolymer is due to the synergistic effect of both polymers: the backbone polymer providing the hydrogen bonds between the hydrogel and mucus layer as well as the adhesive promoter that contributes to the mucoadhesion by increasing the chain interpenetration. Furthermore, oral insulin delivery was investigated using hydrogels of poly (methacrylic acid-g-ethylene-glycol) P(MAA-g-EG) by Peppas et al. [47]. A hypoglycaemic effect combined with insulin absorption was observed in rats. These results could be due to the ability of the complexes to entrap and protect the drugs in their network structure.

At the pH present in the small intestine, these complexes dissociate and the network swells and releases the peptide [47, 48]. Moreover, these hydrogels were shown to decrease the TEER (transepithelial electrical resistance) across the Caco-2 cell models with no sign of cytotoxicity [36]. The in vivo studies were done in male Sprague- Dawley rats using a closed loop absorption method. 25IU/kg of human recombinant insulin was incorporated into the polymer and infused in an isolated ileal segment. The control sample contained the polymer without insulin. At predetermined intervals blood samples were withdrawn from the jugular vein, serum was collected and the insulin levels were determined by enzyme immunoassay. The insulin bioavailability of the formulation was measured using subcutaneous injections. The bioavailability of insulin was shown to increase to 6.2% compared to the control [34]. The mucoadhesion of their delivery system is mediated by weak, non covalent bonds such as hydrogen bonds, van der Waal’s forces and ionic interactions resulting in mucoadhesion that may not be strong enough to localize the hydrogels at the specific site for a sufficiently long time. This suggests that although p (MAA-g-EG) hydrogels are promising carriers for oral insulin delivery the bioavailability of the protein is still too low for the system to be commercialized. Thiolated polymers are another promising class of mucoadhesive with their capability to
form strong covalent bonds through the disulfide binding of the polymers with the mucus gel layer of the mucosa.

**Nanomedicine**

Nanomedicine has been defined as “applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems.”[49] Several nanoparticles, nanoconjugates have been extensively investigated, undergoing regulatory processes and very few of them received regulatory approval. [50-54] Nanomedicine due to their charismatic, unique features attracted a huge scientific community nowadays. The particles below or closer to 200 nm will remain in the circulation for longer periods of time due to size range of spleen fenestrations i.e. 200-500 nm.[55] The particles that are 100-200 nm size range are capable enough to avoid uptake in the liver, but small enough to avoid filtration in the spleen. The active targeting strategies have also been extensively investigated and they are found to be fruitful in the development of very prominent dosage forms [56-59]. PRINT (particle replication in non-wetting templates) technology enables the commercial productions of nanoparticles variable size ranges [60-63]. So many other nanoparticles methods were also established for laboratory practices [64]. Interactions between nanoparticles and biological system is not well developed but various nano material properties such as size, shape, surface chemistry, roughness and surface coatings are some important parameters those are useful in the preliminary predictions [65, 66]. Some of the distinguishing capabilities of nanoparticles in drug delivery are (i) Large payload of drug molecules (ii) protection of drug molecules from degradation (iii) ligand attachment to the nanoparticle surface is possible (iv) bypass multidrug resistance mechanisms like P-gp [50]. Animal model investigations showed that the size range in between 50-200 nm with neutral, slightly negative or positive charged particles can move through the tumour vasculature [67, 68]. Body distribution studies have shown that nanoparticles larger than 230 nm accumulate in the spleen due to the capillary size in this organ [69]. Moreover, less than 200nm particles undergo endocytosis process [70-72]. The self-self and self-non-self interactions will be less with slightly charged particles [50]. If the charge will increases it leads to excessive macrophage scavenging as well as increased clearance by RES. Slightly positive or negative charged particles are suitable
for drug delivery *in-vivo*. Highly charged particles could cause toxicity by interfering with the normal integrity of tissues [73]. Interestingly nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles [74].

Nanoparticles are preferred over micro-particles for drug delivery and other medical applications because nanoscaled materials match the typical sizes of natural functional units in living organisms and allows the nanoparticles to better interact with the biomolecules [75]. The drugs are entrapped inside the nanoparticles so that the amount and type of drug does not affect the pharmacokinetic properties and biodistribution of the nanoparticles [50]. Protein nanoparticles can be easily modified to carry different charges on their surface. This strategy is used for covalent attachment of drugs and other chemicals for targeted drug delivery [76]. Nanoparticles containing proteins on their surface are targeted to cancer cells because proteins can bind to cancer cell surface receptor proteins which are reported to be increased in a wide range of cancer cells [50].

**Drugs used to treat colorectal cancer**

Several drugs or drug combinations are used to treat colorectal cancer. Combination of two or more of these drugs are tried to make them more effective. (i) 5-Fluorouracil (5-FU) and leucovorin (folinic acid). (ii) Capecitabine (Xeloda®), is changed to 5-FU when it gets to the tumor site, (iii) Irinotecan (Camptosar®) to treat advanced colorectal cancer, (iv) Oxaliplatin (Eloxatin®). The side effects of chemotherapy depend on the type and dose of drugs given and the length of time they are taken. General side effects of chemotherapy drugs include: hair loss, mouth sores, loss of appetite, nausea and vomiting, Increased chance of infections (due to low white blood cell counts), easy bruising or bleeding (due to low blood platelet counts), fatigue (due to low red blood cell counts) etc..

Therefore, the herbal drugs which offer high patient compliance and very few side effects have attracted much attention these days for the prevention and treatment of cancer.

**Curcumin and its chemotherapeutic potential**

Turmeric, a common spice to India and the surrounding regions is derived from the rhizome of Curcuma longa. Fractions of turmeric known as curcuminoids (curcumin,
demethoxy curcumin, and bis-demethoxy curcumin) are considered the active compounds. Curcumin is the primary curcuminoid being studied in a host of areas including Alzheimer’s disease, inflammation, chemoprevention, chemotherapy and antioxidant potential. Pre-clinical studies in a variety of cancer cell lines have consistently shown that curcumin possesses anti-cancer activity [77]. *In vitro* studies using colon, gastric, hepatic, leukemia, ovarian, pancreatic, and prostate cancer cell lines have shown that curcumin possesses a potentiating effect on traditional pharmaceuticals such as 5-fluorouracil (5-FU), all-trans retinoic acid, cisplatin, celecoxib, and doxorubicin [78].

**Mechanisms of action of curcumin in prevention of colon cancer** - several mechanisms have been reported in the literature [55, 77-82] and some of the well established findings are as follows:

I. **Inhibition of serine/threonine kinases – protein kinase C (PKC) and c-jun N-terminal kinase (JNK):** Serine-threonine kinases have an important role in the regulation of signalling cascades that control cell proliferation and death and therefore, represent targets for cancer therapy. The action of curcumin to inhibit oncogenesis has been observed at the vicinal thiols on the catalytic domain leading to the inactivation of PKC.

II. **Activator protein-1 (AP-1)-** Curcumin inhibits the AP-1 protein thereby regulating transcriptional factors.

III. **Nuclear factor-kappa B (NF-kB)-** Curcumin decreases the NF-Kb factor thereby inducing apoptosis.

IV. **Early growth response gene product** - Early growth response (Egr-1) gene products modulate the activity of many genes including EGFR. Interruption of the ERK signalling pathway by curcumin (15 lM) led to a reduction in transactivation of Egr-1 as evidenced in Caco-2 and HT-29 cells.

V. **Cyclooxygenase-2 (COX-2)** - Curcumin displays COX-2 inhibition for the chemoprevention of colon cancer.

VI. **Nitric oxide synthase** - Nitric oxide is involved in the expression of COX-2 subsequently leading to the activation of pro-inflammatory prostaglandins, which have been linked to cancer.
VII. **Epidermal growth factor receptor (EGFR)**- Through the inhibition of the Egr-1 by curcumin, a known transcription factor, a reduction has been observed in EGFR expression in Caco-2 and HT-29 cells.

![Curcumin mechanisms in prevention of colon cancer](image)

**Fig 1E: Curcumin mechanisms in prevention of colon cancer [81]**

**Biodegradable polysaccharides for colon cancer**

Several biocompatible and partially biodegradable synthetic macromolecules have been investigated for several biomedical applications. Poly (Lactic Acid) (PLA), Poly (Lactic-co-Glycolic acid) (PLGA) are among the highly preferred polymers in drug delivery due to their ability to fabricate nanoparticle with less PDI and reproducible size parameters and proved non-toxicity [83]. Natural polymer chitosan has been found suitable for nanoparticle preparation to incorporate proteins [84], to deliver cytotoxic agents and
DNA-based drugs at target sites [85, 86]. Non biodegradable metal nanoparticles were demonstrated for magnetic targeting and imaging [87] or delivery [88].

Proteins are better than synthetic polymers for medical applications because they are biocompatible and feasible to modify their surfaces to attach drug molecules, targeting ligand as well as imaging agents [89-91]. The basic functional groups amino and carboxyl groups can be modified to add certain moieties under particular pH conditions. Plant proteins extensively studied to develop nanoparticles, microspheres, hydrogels, and bio-medical engineering purposes. Plant proteins like gliadin, zein, possessing net negative charges and hence are more suitable for delivery of positively charged drugs [92].

**Gliadin**

**Source**

A wide diversity of food has been developed to take advantage of the properties of Wheat kernel proteins which plays a significant role in human diet. These proteins are divided into 2 groups i.e. heterogeneous group of non-gluten proteins (generally about 15-20% of total wheat protein) and gluten protein (generally about 80-85% of total wheat protein) gliadin are believed to act as plasticizers. Gliadin has surface-active properties could be employed in the development of nanoparticles or microparticles, which could act as a vector for delivering certain medicines into target within the human body.

**Chemical structure**

Wheat gluten is a protein carbohydrate complex of which proteins are the major component. Wheat gluten consists of main fractions gliadin and glutenin in which gliadin is soluble in neutral 70% ethanol, made of single chain polypeptides with an average molecular weight of 25-100 kDa, contains intramolecular disulphide bonds and Glutenin an alcohol-insoluble fraction consisting of gliadin-like subunits stabilized by intermolecular disulphide bonds in large aggregates with molecular weight greater than 106 kDa [95]. The term gliadin defines a group of proteins extracted from gluten by 70% ethanol [96]. It is composed of glutamine (about 40%), proline (14%) and phenyl (Fig 1F) [97]. Gliadin is successfully divided into four subfractions: α-(the fastest), β-(the slowest), γ- and ω- in accordance with the mobility in A-PAGE analysis [98, 99]. All
subfractions have extremely low solubility in aqueous solution except at intense pH. The amino acid composition shows that gliadin has equal amounts of polar and neutral amino acids, primarily glutamine (about 40%) in addition to high proline substance (14%) [100]. Gliadins do not have the common drawbacks of synthetic materials due to the presence of initiator residues. As plant proteins, they are known as prion-free unlike animal proteins [101].

![Gliadin Chemical Structure](image)

**Fig 1F: Structure of gliadin molecule**

**Preparation method of gliadin nanoparticulate systems**

The protocol to prepare gliadin nanoparticles can be classified into 2 principal techniques; desolvation [98, 102] and more recent electrospray deposition technique, which is an automated instrumental process [103]. Table 1D summarizes the list of drugs preferably incorporated in the gliadin polymer by using different methods along with their size, zeta potential and remarkable results.

**Desolvation method**

The preparation of gliadin nanoparticles by desolvation process is derived from the coacervation method of microencapsulation. In this method a desolvation factor, such as natural salts or alcohol, should be added slowly to protein solution. By adding this factor, protein structure will changed, when it reaches to a certain level of a desolvation, protein clump will be formed (Fig 1G). This is further stabilised by cross linking agents like gluteraldehyde [117].
Table 1D: Drugs and Model Chemicals Investigated by Using Gliadin

<table>
<thead>
<tr>
<th>S. No</th>
<th>Drug investigated</th>
<th>Method</th>
<th>Size nm</th>
<th>Zeta potential mV</th>
<th>Result</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All trance retinoic acid (vit-A)</td>
<td>Desolvation</td>
<td>464 ±14</td>
<td>-3.5 ± 0.15</td>
<td>Highly stable particles with EE (75%)</td>
<td>[104, 105]</td>
</tr>
<tr>
<td>2</td>
<td>Alpha-tocopherol (vit-E)</td>
<td>Desolvation</td>
<td>900</td>
<td>~0</td>
<td>Sphere shaped particles with EE (77%)</td>
<td>[106]</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin E (hydrophobic), Linanal acetate (polar) Benzalkonium chloride (amphiphilic)</td>
<td>Desolvation</td>
<td>900</td>
<td>-1</td>
<td>Confirmed the strong interaction between gliadins and apolar compounds</td>
<td>[107]</td>
</tr>
<tr>
<td>4</td>
<td>Clarithromycin</td>
<td>Desolvation</td>
<td>250-500</td>
<td>+22.8</td>
<td>Improved bioavailability, colon specific targeting</td>
<td>[108]</td>
</tr>
<tr>
<td>5</td>
<td>Carbazole (lipophilic model drug)</td>
<td>Desolvation, covalent binding of Dolichos biflorus lectin (DBA)</td>
<td>587±35</td>
<td>-</td>
<td>DBA binding to the surface of these carriers provided a greater specificity for colonic mucosa</td>
<td>[109]</td>
</tr>
<tr>
<td>6</td>
<td>Amoxicillin</td>
<td>Desolvation</td>
<td>312 ± 12</td>
<td>26.6±0.8</td>
<td>Eradicated <em>H. pylori</em> from the gastrointestinal tract more effectively than amoxicillin</td>
<td>[110]</td>
</tr>
<tr>
<td>7</td>
<td>(metformin, diclofenac and 5-fluouracil)</td>
<td>Nanofibres developed by electrospinning method</td>
<td>-</td>
<td>-</td>
<td>Increasing loading temperature increases the diffusion coefficient of drugs</td>
<td>[111]</td>
</tr>
<tr>
<td>8</td>
<td>tetanus toxoid</td>
<td>Desolvation method and ovalbumin loading</td>
<td>325</td>
<td>-</td>
<td>Developed for oral immunization as a model</td>
<td>[112]</td>
</tr>
<tr>
<td>9</td>
<td>triple therapy (amoxicillin, clarithromycin and omeprazole)</td>
<td>Desolvation and lectin binding</td>
<td>-</td>
<td>-</td>
<td>triple therapy showed a 94.83% eradication rate</td>
<td>[113]</td>
</tr>
<tr>
<td>10</td>
<td>clarithromycin, and omeprazole</td>
<td>Desolvation</td>
<td>400 to 650</td>
<td>-</td>
<td>Showed greater eradication effect</td>
<td>[114]</td>
</tr>
<tr>
<td>11</td>
<td>acetohydroxamic acid (AHA)</td>
<td>Desolvation and lectin conjugation</td>
<td>-</td>
<td>-</td>
<td>Eradication is 2 folds higher</td>
<td>[115]</td>
</tr>
<tr>
<td>12</td>
<td>superoxide dismutase</td>
<td>Combined with gliadin</td>
<td>-</td>
<td>-</td>
<td>gliadin combined plant superoxide dismutase extract promotes antioxidant property</td>
<td>[116]</td>
</tr>
</tbody>
</table>
In order to obtain dispersed nanoparticles continuous stirring is needed. System turbidity will be increased owing to this desolvation factor. The only stabilizer used in this method is Pluronic F-68, which is non-toxic, easy available and cost effective. Pluronic F-68 is very potent suppressor of carcinogenesis in the colon of rats and mice [118]. This may be a beneficial effect in cancer nanoparticles preparations.

**Electrospray deposition system**

Spray drying is a well established method which is commonly used in the pharmaceutical company for producing a dry powder from a liquid phase. The conventional spray dryer uses rotary atomizers and pressure nozzles, when the viscosity of the liquid is sufficiently low. It has been known that nanofibrous non washable meshes are generated at high concentrations of polymers [119]. The electrospray deposition system generates a plume of droplets by charging the liquid at a high voltage and the charged droplets are sprayed from the tip of the nozzle [120]. The new Nano spray Dryer utilizes a vibrating mesh technology for fine droplets production [121]. To overcome the drawbacks of desolvation method Muhammad.G and co-workers applied use of liquid atomization by means of electrical forces. This method has several advantages such as producing of smooth special nanoparticles having size 218.66 ± 5.1 and drug loading efficiency 72.02 ± 5.6 %. was achieved without using any surfactants. Fig 1H illustrates the functional principal of electrospraying system which is similar to the electrospinning process.

![Fig 1G: Preparation of gliadin nanoparticles with desolvation method [98].](image)
Polysaccharides as a polymer for nanocarriers

Polysaccharides have received increasing attention because of their outstanding physical and biological properties [122]. Chitosan, [Fig 1I] a linear amino polysaccharide composed of randomly distributed (1>4) linked D-glucosamine and N-acetyl-D-glucosamine units, is obtained by the deacetylation of chitin, a widespread natural polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp [123]. This cationic polysaccharide has drawn much attention within pharmaceutical and biomedical applications, owing to its abundant availability, unique mucoadhesivity, inherent pharmacological properties, and other beneficial biological properties such as biocompatibility, biodegradability, nontoxicity and low-immunogenicity [123-125]. The physicochemical and biological properties of chitosan are greatly immanent by its molecular weight and degree of deacetylation. Detailed characteristics of chitosan for biomedical applications are well described in several widespread reviews [125].

Fig 1I: Structure of chitosan molecule
Cancer-targeted drug delivery using chitosan and its derivatives

The serious tailback of conventional cancer chemotherapeutics includes high toxicity of most anticancer drugs, due to unsystematic distribution of drugs towards disease and healthy cells following systemic administration. In addition, anticancer drugs often suffer from poor solubility in water and thus need to use organic solvents or detergents for clinical applications, resulting in undesirable side effects such as venous irritation and respiratory distress [126]. Therefore, designing a distinct carrier system that encapsulates a large quantity of drugs and specially targets tumor cells is indispensable for successful cancer therapy.

Chitosan is an ideal natural polymer for the design and development of drug delivery systems structured at micro and nanoscopic scales. Particularly, its muco-adhesiveness [116-117], biocompatibility [118-119] and capacity to promote the absorption of poorly absorbable macromolecules across epithelial barriers by transient widening of cell tight junctions thus modifying the parallel transport [120-123] have been exploited in the development of nanocarrier systems for transmucosal delivery. A recognized feature of chitosan-based nanostructured systems is their capacity to protect sensitive therapeutic macromolecules against degradation and their ability to overcome mucosal barriers. As a consequence, their application has been centred particularly in non-invasive routes of administration including transmucosal administration of proteins [124-128] and genetic material [129-133].

Conjugation of folate

![Fig 1J: Structure of folic acid molecule](image)

Folic acid (FA) is appealing as a ligand for targeting cell membrane and allowing nanoparticle endocytosis via the folate receptor (FR) for higher transfection yields. It is a stable, inexpensive, and poorly immunogenic chemical with a high affinity for the FRs [127, 128]. Because the FRs is over expressed on many human epithelial cancer cells, the conjugation of drugs and macromolecules with folic acid can improve their uptake and targeting ability [129-131].
Importantly, the high affinity of folate to bind its receptor (1 nM) [132] and folate’s small size allows its use for specific cell targeting. Moreover, the ability of FA to bind its receptor to allow endocytosis is not altered by covalent conjugation of small molecules. [133-134]. Hence, folate conjugation to the surface of chitosan and chitosan derivatives-based nanoparticles has been one of the actively studied strategies to vectorized drugs over the past few years [131, 134-136]. These systems have been originated with a view to achieve targeting effect in the delivery of cytostatic drugs to tumor cells, genetic material, or anti-arthritis therapies and also for diagnostic and imaging purposes. To this end, the majority of in vitro studies have been conducted in various types of cell lines well known to over-express the human FRs (FRa and FLRb), such as HeLa [137], HT29 [136], Caco-2 [136], B16F1[138], KB [139, 140], HepG-2 [141] and SKOV3 [135, 142] cells. The evidence from most of these studies is consistent to indicate that the folic acid modification promotes the uptake of nanoparticles by FRs-positive tumor cell lines most likely via receptor-mediated endocytosis, but has little impact on other cells without FRs [143]. Results of transfection studies showed that folate-chitosan-based nanoparticles enhanced the reporter gene expression against a cell line over-expressing FR (SKOV3 cells) compared to a FR-deficient cell line (A549 cells) and did not induce obvious cytotoxicity against HEK 293 cells [142]. In turn, Nanoparticles made out of folated-grafted chitosan has been produced to transfect interleukin-1 receptor antagonist (IL-1Ra) in synovial mononuclear cells and CD14+ cells via the targeting of the folate receptor-b [144]. Compared to unmodified chitosan or naked DNA, this system allowed for enhance in IL-1Ra expression combined with a diminution of cytotoxicity in vitro, and reinforced protection against inflammation and abnormal bone metabolism in vivo.

Colon-Specific Drug Delivery Systems
During the last decade there has been increased interest in developing site-specific formulations for targeting into the colon through oral route. Oral delivery intended for targeted drug release into the colon is attractive for localized topical treatment of pathologies mainly constipation, diarrhoea, inflammatory bowel diseases (ulcerative colitis and crohn’s disease), colon cancer and infections. Apart from local treatment, the colon can also be utilized for the purpose of systemic therapy [145]. Treatment might be more effective if the therapeutic drugs delivered in its intact form as possible to the targeted site. Therapeutics drugs needed to be protected from absorption and hostile environment of upper GI tracts are best suitable for colonic delivery due to longer
residence time, highly responsive to absorption enhancer and lower luminal and mucosal digestive enzymes[146].

It offers an opportunistic site for oral delivery of various candidates including proteins and peptide along with cytokine inhibitors and antibiotics which normally inactivate by the formidable barriers of stomach and small intestine [147]. Additionally colon is rich in lymphoid tissues which facilitates for efficient local production of antibodies for vaccine delivery. Other drugs being used for potential delivery includes vermicides, diagnostic agents, erythroprotein, contraceptive peptides and prophylaxis of colon carcinomas rhythm e.g., asthma, arthritis, rheumatic disease, ulcer, ischemic heart disease and hypertension.

Colonic delivery can be accomplished by oral or rectal administration. Rectal administration offers the shortest route to targeting drugs in the colon. The rectal route has traditionally been used to administer medicaments in the form of suppositories and enemas to the distal guts, although such formulations rarely succeeded in spreading beyond the descending colon. Also, the rectal route is not convenient or acceptable for most patients and hence the oral route is the preferred route of drug administration. However, colonic drug delivery via the oral route is not without its challenges. The colon constitutes the most distal segments of the GI tract and so on orally administered formulations must retard drug release in the upper gastrointestinal regions but release the drug promptly on entry into the colon ¹³. There are several ways in which colon specific delivery has been attempted may be broadly divided into four types, which are

1. Time-dependent (or timed release) system designed to release drug after a predetermined time [148].
2. pH-dependent (or delayed release) system designed to release drug in response to change in pH [146].
3. Microbially-dependent (or microbially controlled) system designed to make use of abundant enterobacteria in the colon.
4. Prodrug-dependent system based on cleavage of the link between drug and carrier via reduction and hydrolysis by enzymes from colon bacteria.

Pressure-dependent system making use of luminal pressure of the colon. Effective treatment of colon cancer by conventional therapy requires relatively large doses to compensate drug loss during its passage through upper GI tract, which may be associated with the risk of undue side effects. This can be overcome by site-specific delivery of the drug molecule to colon for
effective localization of pharmacologically active moiety at pre-identified target in therapeutic concentration, while restricting is access to non-target normal cellular linings, thus minimizing toxic effects and maximizing therapeutic index.

In order to achieve successful colonic delivery via the oral route is not without challenge. As colon constitutes the distal segment of GI tracts and so the formulations administered orally must retard drug release in vicinity of stomach and small intestine environment but simultaneously prompt release upon entry into the colon. Retardation and/ or delaying of drug release is not easily achieved since the dosage form may breakdown when subjected to diverse physical and chemical assaults of upper GI tracts. While the gradual changes in physiological parameters in colon can be characterized by low fluid environment, viscous nature of luminal content and decrease in enzymatic activity and motility may hinder the dissolution and drug release from the formulation.

Overall, the system necessitates the triggering elements that can resist/ respond to the sudden dramatic physiological changes of colon in order to affect for the performance of the delivery systems. However, the resident colonic microfloras through metabolic degradation and on increasing pH gradient may affect the drug release have been extensively uses as triggering elements for the purpose of achieving colon specific targeting. Moreover the resident colonic microfloras may affect the drug release through metabolic degradation. Targeting of intact drugs to colon can be obtained by utilizing thicker enteric coating layers and/ matrices containing slow releasing biodegradable polysaccharides.

Earlier various enteric polymers have been widely used to form films in connection with formulations intended to target drug delivery on the colon. Site specificity of such formulation has, however, usually been poor. Therefore in the present study it has been decided to determine whether better single-unit formulations for colon specific drug delivery could be prepared using enteric polymers to form coating films. A secondary aim of the work described here has been to improve site-specificity in relation to the colon through use of guar gum as formulation matrix excipients. Incorporation of guar gum has been intended to delay dissolution (time even where the pH in the gastrointestinal tract exceeded 7) and degraded specifically by colonic bacteria, which holds great promises. The guar gum matrix remain intact due to the fact that they are resistant to the hostile environment of upper GI tract but once they reach in the colon, they are acted upon by the bacterial bioenvironmental and the result in the degradation of the matrices.
and finally release the drug. It has been hoped that it would be possible to prevent drug liberation and absorption in the terminal ileum and defer commencement of drug absorption until the proximal colon was reached. Hence it is proposed to develop and characterize a colon targeted drug delivery system bearing potent methotrexate and 5-Fluorouracil anti-cancer drugs using pH-sensitive Eudragit R polymer, hydrophilic HPMC and natural biodegradable guar gum polysaccharide to deliver the drug extensively to the colon to control colo-rectal cancer disease without drug loss in the upper GI tract.

**Drug release based on variation of pH**

The most important physiological factor considered in the design of colonic formulations is pH gradient of the GI tract. In the stomach pH changes between 1 and 2 during fasting but increases after eating [149]. The pH is progressively increasing in normal healthy subjects of about 6.6 ± 0.5 in the duodenum and about 7.5 ± 0.4 in the terminal ileum²². From the ileum to the colon pH declines significantly of about 6.4 ± 0.4 and then a slow rise from right to the left colon with a final values of 7.0 ± 0.7. However pH values as low as 5.7 have been measured in the ascending colon in healthy volunteers [150]. The pH in the transverse colon is 6.6 in the descending colon 7.0. This pH differential between the stomach and small intestine has historically been exploited to deliver drugs to the terminal ileum/colon by way of pH-sensitive enteric coatings with relatively high threshold pH for dissolution. Coating approach is one of the simplest formulation technologies offers significant advantages in terms of cost and ease of manufacture. From formulation standpoint, coated dosage forms may be either single unit or a multiparticulate system, and of these may be single-layer product or multi-layered products.

Solid formulations for colonic delivery that are based on pH-dependent drug release mechanism are similar to conventional enteric-coated formulations but they differ in target site for delivery and therefore type of enteric polymers. In contrast to conventional enteric coated formulations colonic formulations are designed to deliver drugs to the distal (terminal) ileum and colon, and utilize enteric polymers that have relatively higher threshold pH for dissolution. Most commonly used polymers are derivatives of acrylic acid and cellulose. These polymers have ability to withstand an environment from low pH (1.2) to neutral pH (7.5) for several hours.

Use of pH-dependent polymers is based on these differences in levels. The polymers described as pH-dependent in colon specific drug delivery are insoluble at low pH levels but become increasingly soluble as pH rises. There are various problems with this approach. The pH in the
GI tract varies between and within individuals depends on diets and diseases. During acute stage of inflammatory bowel disease colonic pH has been found to be significantly lower than normal. In ulcerative colitis pH values between 2.3 and 4.7 have been measured in the proximal parts of the colon [151]. Although a pH-dependent polymer can protect a formulation in the stomach and proximal small intestine, it may start to dissolve even in the lower small intestine, and the site specificity of formulations can be poor. 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; nevertheless there are some generally accepted GI residence values for various parts of GIT. The critical factor that influences the performances of the polymers is the pH value at which dissolution occurs. Polymers with ionizable phthalic acid group dissolve much faster and at a lower pH than those with acrylic or methacrylic acid groups. The presence of plasticizer [152] and the nature of the salt in the dissolution medium also influence the dissolution rate of Eudragit R [153]. Colon targeted drug delivery systems based on methacrylic resins has been described for insulin [154], prednisolone [155], quinolones [156], salasazine [157], cyclosporine [158], beclomethasone dipropionate and naproxane [159].

Eudragit™ products are pH-dependent methacrylic acid polymers containing carboxyl groups. The number of esterified carboxyl group’s affects the pH level at which dissolution takes place. Eudragit™ S is soluble above pH 7.0 and Eudragit™ L above 6.0. Eudragit™ S coatings protect well against drug liberation in the upper parts of GI tract and have been used in preparing colon-specific formulations. Eudragit™ S coatings have been used to target the anti-inflammatory drug 5-aminosalicylic acid (5-ASA) in single unit formulations on large intestine. Eudragit™ L coatings have been used in single unit tablets to target 5-ASA on the colon in patients with ulcerative colitis or crohn’s disease. The polypeptide hormone vasopressin and insulin have been administered to rats orally in Eudragit™ S-coated single-unit capsules. Eudragit™ S-coated insulin capsules have also been administered orally to hyperglycaemic beagle dogs. Eudragit™ S has also been used in combination with another methacrylic acid copolymer, Eudragit™ L, in colon-targeted systems to regulate drug delivery. Dissolution studies showed that drug release profiles from enteric-coated single-unit tablets could be altered in vitro by changing the ratios of the polymers, in the pH range 5.5 to 7.0.

Numerous Eudragit R coated oral dosage forms of salsalazine are currently in use for treatment of ulcerative colitis and crohn’s disease. Morishita et al [45] compared the insulin delivery of
two formulations containing Eudragit R L-100 and Eudragit R LS respectively. The disadvantage of this technique is the lack of consistency in the dissolution of polymer at desired site. Depending on the intensity of the GI motility, the dissolution of the polymer can be in the distal portion of the colon or at the end of the ileum. Moreover, many factors such as the presence of short chain fatty acids, residues of the bile acids, carbon dioxide or other fermentation products reduce the colonic pH to approximately 6 and call its pH as a trigger into question. Eudragit R S, a model pH dependent polymer was used to coat rapidly disintegrating tablets. The tablets were administered to healthy volunteers and studied for their in vivo behaviours using gamma scintigraphy. Though the polymer coat protected the tablet during its passage through the stomach and upper small intestine, its site specificity was poor. The disintegration sited varied from ileum to the splenic flexure.
<table>
<thead>
<tr>
<th>Enteric polymers</th>
<th>Optimum pH for dissolution</th>
</tr>
</thead>
</table>
| Methacrylic acid copolymer, Type A  
(Eudragit R L-100* & Eudragit R L 12,5) | ≥6.0 |
| Methacrylic acid copolymer, Type B  
(Eudragit R S-100*Eudragit R S12,5) | ≥7.0 |
| Methacrylic acid copolymer, Type C (Eudragit R L-100-55*)  
Methacrylic acid copolymer dispersion (Eudragit R L-30D-55*) | ≥5.0 |
| Eudragit R FS-30D** | ≥7.0 |
| Shellac (MarCoat 125*** & 125N***),  
Hydroxylpropylmethylcellulose acetate succinate (HPMCPAS) | 7.0 |
| LF Grade | ≥5.0 |
| MF Grade | ≥6.0 |
| HF Grade  
Hydroxylpropyl methylcellulose phthalate (HPMCP) | ≥6.8 |
| HP-55 &HP-55S | ≥5.5 |
| Cellulose acetate phthalate (CAP); (Aquatic R**) | 6.0 |
| Polyvinyl acetate phthalate (PVAP); (Coateric R**) | 5.0 |
| Cellulose acetate trimelliate (CAT) | 5.5 |