CODESTM DRUG DELIVERY: CAPECITABINE IN THE MANAGEMENT OF COLORECTAL CANCER

A Dissertation Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

Chennai-600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

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October 2017

CERTIFICATE

This is to certify that the dissertation entitled **"CODESTM DRUG DELIVERY: CAPECITABINE IN THE MANAGEMENT OF COLORECTAL CANCER"** is a bonafide work submitted by **University Reg. No. 261511110,** to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for **Master of Pharmacy** in **Pharmaceutics** and has been conducted under the guidance of **Dr. S. Subramanian, M.Pharm, Ph.D.,** Department of Pharmaceutics, PSG College of Pharmacy, Peelamedu, Coimbatore in the academic year of 2016-2017.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"CODESTM DRUG DELIVERY: CAPECITABINE IN THE MANAGEMENT OF COLORECTAL CANCER"** submitted by **University Reg. No. 261511110** is a bonafide work carried out by the candidate under the guidance of Dr.S.Subramanian, M.Pharm, Ph.D., and submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2016-2017.

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Dedicated To

My Parents,

Respectful Guide

&

Almighty

ACKNOWLEDGEMENT

Behind every achievement there are many helping hands that aid in reaching the ultimate goal.

I consider it is a great honour to express my deepest sense of gratitude and indebtness to my beloved guide, **Dr. S. SUBRAMANIAN., M. Pharm., Ph.D.,** who was guiding me at every stage of the project and for keeping me in high spirit. His innovative and constructive ideas have been very invaluable, which made the work interesting and easy.

I would like to express my sincere gratitude to my generous Principal, **Dr. M. RAMANATHAN., M. Pharm., Ph.D.,** for providing the facilities from time to time for the completion of this work.

I owe my most sincere gratitude to **PSG Sons and Charities** for providing all the facilities to carry out this work.

I would like to thank the head of the department of pharmaceutics **Dr. V. Sankar, M.Pharm, Ph.D.,** and rest of my teachers **Mr. S. M. Habibur Rahman., M.Pharm, (Ph.D)., Mrs. Nithya., M. Pharm., Mrs. Vijayalakshmi, M.Pharm., Mr. Vaiyana Rajesh, M.Pharm.,** and **Mr. S. Kartikeyan, M.Pharm., Department of Pharmaceutics**, for their constructive and valuable ideas for the completion of my project work

I express my sincere thanks to my seniors, **Mr. Siram Karthik,** and **Mr. K. Arjunan,** Research Scholar, Department of Pharmaceutics, for his constant advice and support during the whole period of study.

I am thankful to all my friends, who directly or indirectly helped me during my work..

I would like to thank **Mr. Karthikeyan, Mrs. Nithya,** and **Mrs. Jagadeshwari** for helping me in providing various chemicals and glass wares during the project.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CRC	–	Colo Rectal Cancer
CS	–	Chitosan
TPP	–	Tri Poly Phosphate
SEM	–	Scanning Electron Microscopy
PCM	–	Phase Contrast Microscopy
RPM	–	Rotations Per Minutes
TP	–	Thymidine Phosphorylase
GIT	–	Gastro Intestinal Tract
IBD	–	Inflammatory Bowel Diseases
API	–	Active Pharmaceutical Ingredient
MTT assay	–	Microculture Tetrazolium assay
EE	–	Entrapment Efficiency
PSA	–	Particle Size Analyzer
TS	–	Thymidylate Synthase
UTP	–	Uridine Tri Phosphate

INTRODUCTION

Cancer is a general term used to refer to a condition where the body cells begin to grow and reproduce in an uncontrollable way. These cells can then invade and destroy healthy tissue, including organs. Cancer sometimes begins in one part of the body before spreading to other parts. Cancer is a common condition and a serious health problem. More than one in three people will develop some form of cancer during their lifetime. There are hundreds of different types of cancer. In 2012 about 14.1 million new cases of cancer occurred globally. It caused about 8.2 million deaths or 14.6% of human deaths. The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer. In females, the most common types are breast cancer, colorectal cancer, lung cancer and cervical cancer. Treatment for cancer will depend on many factors. Such as the stage and location of the disease it includes the surgery, chemotherapy, radiation therapy. Possible sign and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes over 100 types of cancers affect humans. Cancers harms the body when altered cells divide uncontrollably to form lumps or masses of tissue called tumors. Tumors can grow and interfere with the digestive, nervous and circulatory system.

Tumors form when two things occur

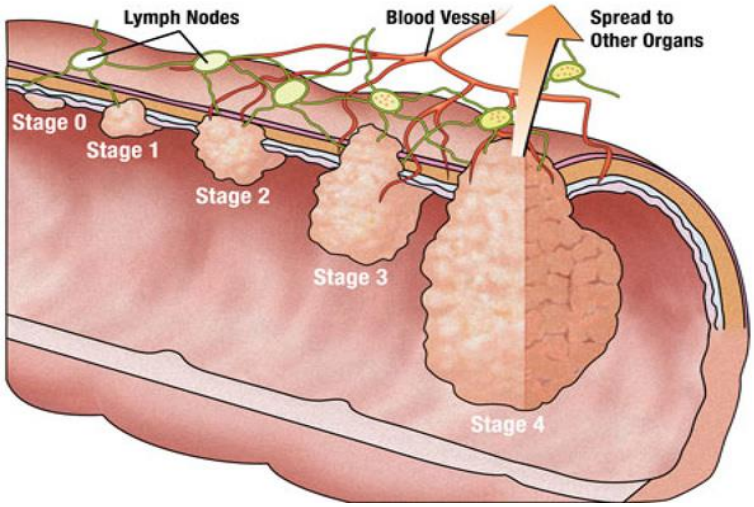
- A cancerous cell manages to move throughout the body using the blood or lymphatic systems, destroying healthy tissue in a process called invasion.
- That cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis.

Colon cancer is one of the three most common cancers diagnosed in the United States and includes cancers of the colon and rectum. In 2007, approximately 1,53,760 new cases were diagnosed and an estimated 52,180 deaths occurred, making colon cancer the second leading cause of cancer-related deaths in the United States. Patient prognosis is determined primarily by the extent or stage of disease, with most patients with early-stage disease being cured. Treatment of colon cancer includes surgery, radiation, chemotherapy, and new targeted molecular therapies.

Colon specific delivery:

The colon drug delivery has a number of important implications in the field of pharmacotherapy. Various diseases including Inflammatory Bowel Diseases (IBD) can be effectively treated by the local delivery of drugs to the large intestine. The treatment of IBD with anti-inflammatory drugs is particularly improved by their local delivery to the bowel. By this technique, absorption of drugs from the stomach and small intestine can be minimized until the drug reaches the large intestine. Various drug delivery systems have been designed that deliver the drugs quantitatively to the large bowel and subsequently to trigger the release of active drug. The treatment of large intestine disorders, such as Crohn's disease, irritable bowel syndrome, colitis, colon cancer and local infectious disease where a high concentration of active drug is needed, can be improved by colon specific drug delivery systems employing various mechanism of release. Moreover, the introduction of several peptide-based drugs in the recent past has triggered the demand to explore alternative non-parenteral routes for their delivery. (Manish p patel et al)

Figure 1: COLON RECTAL CANCER



The site-specific delivery of the drugs to the target receptor sites has the potential to reduce the side effects and improve the pharmacological response. However, for successful colonic drug delivery, many physiological barriers must be overcome, the major one being absorption or degradation of the active drug in the upper part of the GI tract. The disease state can also potentially alter the delivery and absorption characteristics of drug from the colon.

The colon drug targeting is also exploited for systemic delivery of active drugs. Most of the peptide and protein drugs are unstable in the stomach and upper part of intestine. Apart from stability problems, peptides are not well absorbed from the lumen of the GIT due to their large molecular size and highly sensitive to brush border peptidase activity. Comparatively, proteolytic activity of colon mucosa is much less than that observed in the small intestine. Colon specific drug delivery systems protect peptide drugs from hydrolysis and enzymatic degradation in the duodenum and jejunum, and eventually release drugs in the ileum or colon, which leads to greater systemic bioavailability. The specific release in the colon also affects a time delay between administration and onset of action, which can be useful for diseases with various degrees of severity, such as asthma and arthritis.

Various colon specific drug delivery systems are being developed, by taking advantage of the luminal pH in the ileum and the microbial enzymes in the colon, such as pectinase, amylase, dextranase, glycosidase and azoreductase.

Table 1: COLON TARGETING DISEASE, DRUGS AND SITES:

Target sites	Diseases conditions	Drugs and active agents
Topical action	Inflammatory Bowel Disease, Irritable bowel disease and Crohn's disease.	Hydrocortisone, Budenoside, Prednisolonr, Sulfasalazine, Olsalazine, Mesalazine and Balsalazide.
Local action	1)Chronicpancreatitis, pancreatactomy and cystic fibrosis. 2)Colorectal cancer	Digestive enzyme supplements. 5-Flourouracil
	1)To prevent gastric irritation 2)To prevent first pass metabolism of orally ingested drugs 3)Oral delivery of peptides 4)Oral delivery of vaccines	NSAIDs Steroids Insulin Typhoid

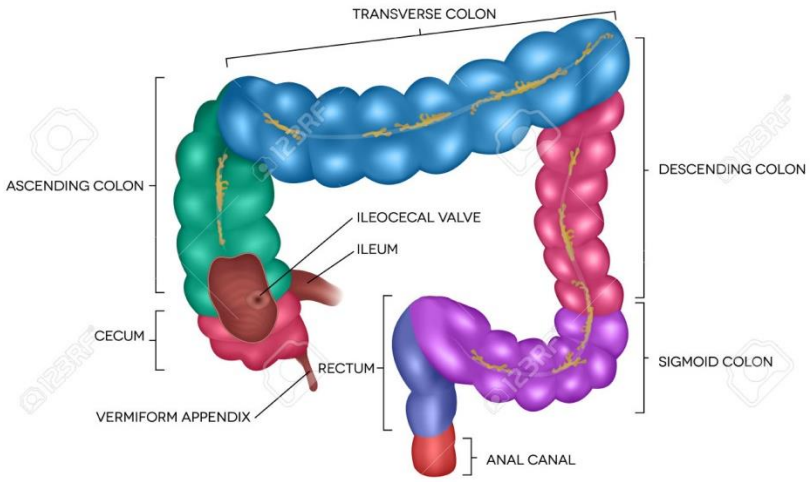
ANATOMIC AND PHYSIOLOGICAL CONSIDERATIONS:

In GIT, large intestine starts from the ileocecal junction to the anus with a length of about 1.5m (adults) and is divided in to three parts, viz., colon, rectum and anal canal.

Colon:

The colon consists of cecum, colon ascendens, colon transvesale, colon descendens and sigmoid colon. Colon is made up of four layers, serosa, muscularis externa, sub mucosa and mucosa.

Figure 2: ANATOMY OF COLON AND RECTUM



The serosa is the exterior coat of the large intestine and consists of areolar tissue that is covered by a single layer of squamous mesothelial cells.

The muscularis externa is composed of the major muscular coat of inner circular layer of fibers that surrounds the bowel and an outer longitudinal layer. Colonic longitudinal muscle fibers are composed of three flat long bands called taeniacoli, which is shorter than other coats of the colon and hence results in wall contractions and formation of haustra.

The sub mucosa is the layer of connective tissue that lies immediately beneath the mucosa.

The mucosa lines the lumen of the colon and is divided into epithelium, laminapropria and muscularis mucosae. The mucosa has a layer of smooth muscles and seperates the sub mucosa from the lamina propria, which supports the epithelium. The blood capillaries and lymphatic vessels supply bio fluids to lamina propria. (Cherukuri Sowmya et al)

- Cecum(proximal right colon)
  6 x 9 cm pouch covered with peritoneum
- Appendix
  A vermiform diverticulum located in the lower cecum
- Ascending colon
  20-25 cm long, located behind the peritoneum
- Hepatic flexure
  Lies under right lobe of liver
- Transverse colon
  Lies anterior in abdomen, attached to gastro colic ligament
- Splenic flexure
  Near tail of pancreas and spleen
- Descending colon
  10-15 cm long, located behind the peritoneum
- Sigmoid colon
  Loop extending distally from border of left posterior major psoas muscle
- Recto sigmoid segment
  Between 10 and 15 cm from anal verge

The adult colon is lined by at least eight distinct epithelial cell types, viz. Columnar and absorptive cells, deep crypt secretary cells, vacuolated cells, micro fold or ‘M’ cells, undifferentiated crypt cells, multi vesicular or caveolated cells, and variety of entero endocrine cells. They constitute a structural and functional barrier protecting the internal milieu from direct contact with luminal components such as the indigenous bacterial flora or exogenously introduced antigens or viruses. The villi of the columnar cells contribute to the surface area of the colon. Apart from stomach, the GIT has normal bacterial flora, which inhibits the growth of other organisms. Some species of normal micro flora produce short chain fatty acids or

antibiotics such as “clostin”,which prevents the growth of pathogens by competing with them for nutrients. The mucous lining of GIT forms a barrier against bacterial invasion of the gut wall. Antibodies Ig A and Ig G enhance the phagocytosis of GI bacteria.

Rectum:

12 cm long; upper third covered by peritoneum; no peritoneum on lower third which is also called the rectal ampulla. About 10 cm of the rectum lies below the lower edge of the peritoneum (below the peritoneal reflection), outside the peritoneal cavity.

Anal canal:

Most distal 4-5 cm to anal verge.

Colorectal polyps:

Colorectal cancer most often begins as a polyp, a noncancerous growth that may develop on the inner wall of the colon or rectum as people get older. If not treated or removed, a polyp can become a potentially life-threatening cancer. Recognizing and removing precancerous polyps can prevent colorectal cancer. There are several forms of polyps. Adenomatous polyps, or adenomas, are growths that may become cancerous and can be found with a colonoscopy. Polyps are most easily found during colonoscopy because they usually bulge into the colon, forming a mound on the wall of the colon that can be found by the doctor. About 10% of colon polyps are flat and hard to find with a colonoscopy unless a dye is used to highlight them. These flat polyps have a high risk of becoming cancerous, regardless of their size. Hyperplastic polyps may also develop in the colon and rectum. They are not considered precancerous.

Risk factors for colorectal cancer include:

- Ulcerative colitis, sometimes called pan ulcerative colitis.
- Familial or multiple polyposis: a disease occurring in some families that consists of multiple adenomatous polyps of the colon which have high malignant potential. Also called familial polyposis colonae, polyposis coli, familial intestinal polyposis, hereditary gastrointestinal polyposis, multiple familial polyposis, Gardner's syndrome, Peutz-Jaegher's syndrome, Canada - Cronkhite syndrome, or Turcot syndrome.

- Crohn's disease: a benign chronic granulomatous inflammatory disease of any or all parts of the colon.
- Diet that is low in fiber, causing digestion to be slowed and transit time through the bowel to be increased.
- Patient history of colon or rectal cancer.
- Patient history of colon or rectal polyps.
- Family history of colorectal cancer or female genital cancer.

Signs and symptoms:

The early signs of colorectal cancer can mimic symptoms caused by other gastrointestinal illnesses, such as influenza, ulcers, and colitis (an inflammation of the colon). If any of the following symptoms last more than two weeks, however, it is important to see a physician for an evaluation:

- Unexplained persistent diarrhea or constipation.
- Blood in or on the stool (can be bright red or very dark).
- Narrower stools than usual.
- Unexplained iron deficiency anemia.
- Intermittent abdominal pain.

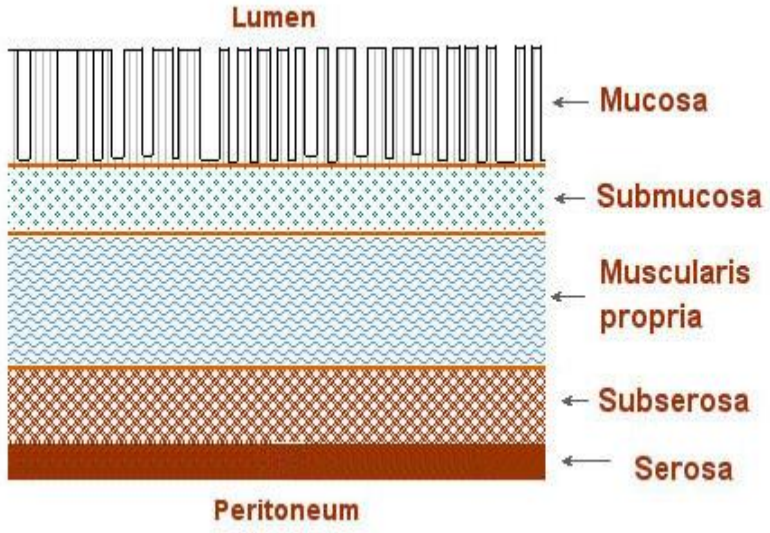
The following symptoms can be signs of rectal cancer:

- Blood in the stool.
- Diarrhea.
- A sense of bowel movement urgency.
- Feeling of inadequate emptying of bowel.
- Excessive straining to have a bowel movement without passing of stools.

Layers of Bowel Wall

- Lumen (interior surface of colon "tube")
- Mucosa
- Surface epithelium
- Lamina propria or basement membrane—dividing line between in situ and invasive lesions
- Muscularis mucosae
- Sub mucosa—lymphatics; potential for metastases increases
- Muscularis propria
- Circular layer
- Longitudinal layer—in three bands called taenia coli
- Sub serosa—sometimes called pericolic fat or sub serosal fat
- Serosa—present on ascending, transverse, sigmoid only (also called the visceral peritoneum)
- Retroperitoneal fat (also called pericolic fat)
- Mesenteric fat (also called pericolic fat)

Figure 3: LAYERS OF BOWEL WALL



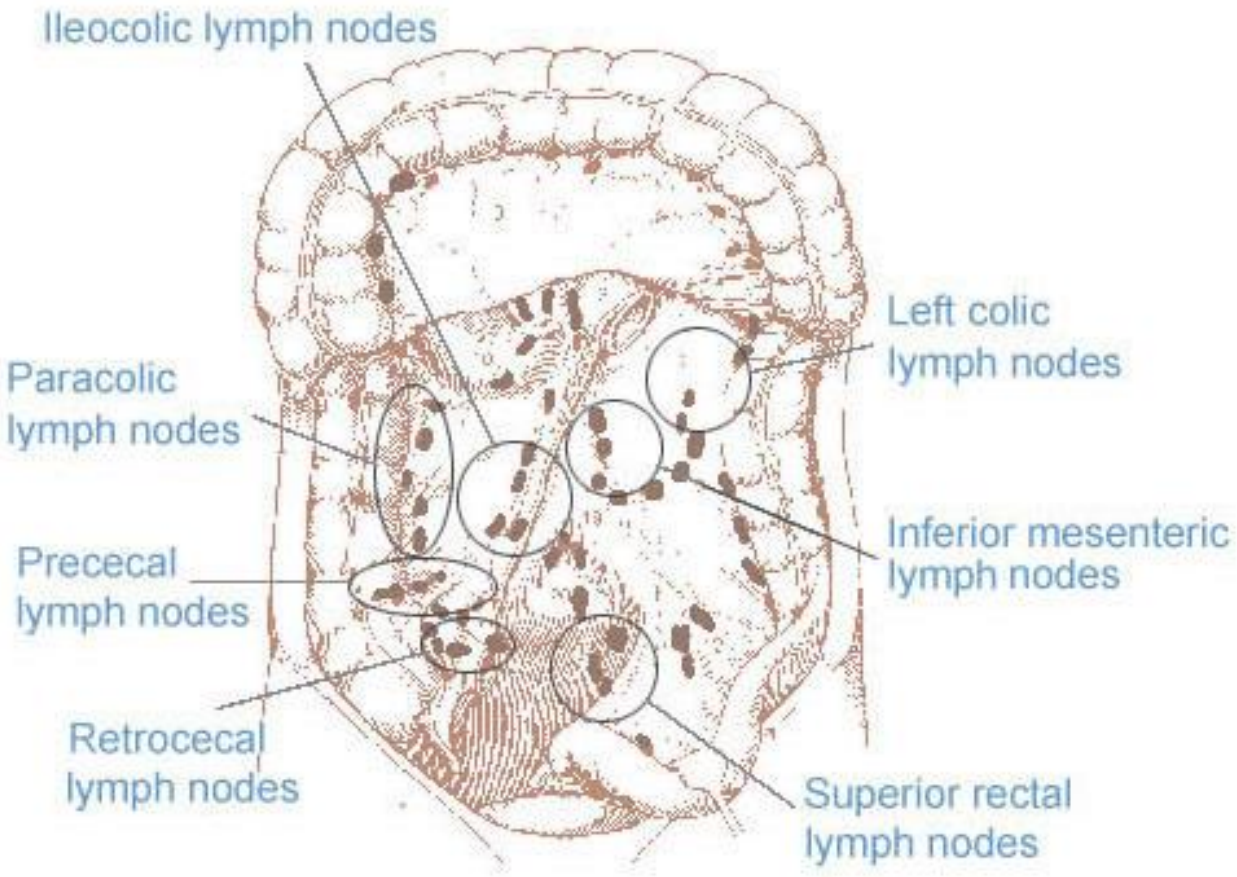
Regional Lymph Nodes:

There are between 100 and 150 lymph nodes in the mesentery of the colon. Regional lymph nodes are the nodes along the colon, plus the nodes along the major arteries that supply blood to that particular colon segment. Lymph nodes along a "named vascular trunk" are those along a vein or artery that carries blood to a specific part of the colon, for example, the inferior and superior mesenteric arteries, sigmoidal artery, left or right colic artery.

Table 2: REGIONAL LYMPH NODES

Segment	Regional Lymph Nodes
Cecum	Pericolic, anterior cecal, posterior cecal, ileocolic, right colic
Ascending colon	Pericolic, ileocolic, right colic, middle colic
Hepatic flexure	Pericolic, middle colic, right colic
Transverse colon	Pericolic, middle colic
Splenic flexure	Pericolic, middle colic, left colic, inferior mesenteric
Descending colon	Pericolic, left colic, inferior mesenteric, sigmoid
Sigmoid colon	Pericolic, inferior mesenteric, superior rectal, superior hemorrhoidal, sigmoidal, sigmoid mesenteric
Rectosigmoid	Perirectal, left colic, sigmoid mesenteric, sigmoidal, inferior mesenteric, superior rectal, superior hemorrhoidal, middle hemorrhoidal
Rectum	Perirectal, sigmoid mesenteric, inferior mesenteric, lateral sacral, presacral, internal iliac, sacral promontory superior hemorrhoidal, inferior hemorrhoidal
Anus	Perirectal, anorectal, superficial inguinal, internal iliac, hypogastric, femoral, lateral sacral

Figure 4: REGIONAL LYMPH NODES



Types of cancer in the colon and rectum:

Here is an overview of the types of cancer that can start in the colon and rectum:

- Adenocarcinoma**.** The most common type of colorectal cancer. More than 95% of colorectal cancers are adenocarcinoma. This cancer starts in the lining of internal organs. The tumors start in gland cells that release, or secrete, fluids.

Other types of cancer that can start in the colon or rectum are much less common:

- Gastro intestinal stromal tumor **(GIST).** These tumors start in special cells in the wall of the digestive tract. They may be found anywhere in the digestive tract. But they rarely appear in the colon. They may be benign, or not cancer, at first. But many do turn into cancer.
- Lymphoma**.** This cancer starts in a type of immune cell called a lymphocyte. Lymphomas often start in bean-sized groups of lymphocytes, called lymph nodes. But they can also start in the colon, rectum, or other organs.
- Carcinoid**.** This cancer starts in special hormone-making cells in the intestine.
- Sarcoma**.** These tumors start in blood vessels, muscle, or connective tissue in the colon and rectum wall.

Factors governing the colon drug delivery:

Physiological Factors:

1. Gastro intestinal transit:

The drug delivery systems first enter in to stomach and intestine via mouth and then reach colon. The nature and pH of gastric secretions and gastric mucosa influence the drug release and absorption. In order to successfully reach colon in an intact form, the drug delivery systems should surpass the barriers in the stomach and small intestine.

In fasted state, the motility proceeds through four phases occurring in stomach and small intestine that span over a period of 2-3h. Phase 1 is a quiescent period of 40-60min. Phase 2 consists of intermittent contractions for a period of 40-60min. Phase 3 is a period of intense contractions sweeping material out of the stomach and down the small intestine followed by Phase 4 with contractions dissipating. The feeding state affects the normal pattern by irregular contractile activity. (Kothawade P D et al).

2. Small intestinal transit:

Normally, the small intestinal transit is not influenced by the physical state, size of the dosage form and the presence of food in the stomach. The mean transit time of the dosage form is about 3-4h to reach the ileocecal junction and the time period is consistent. The dosage form is

exposed to enzymes such as esterase, lipase, amylase, protease, nuclease and brush border enzymes present in the small intestine. The release of drugs from the pro drug based systems and stability of peptides can be affected by bacterial contents in the ileum.

3. Colonic transit:

The bioavailability of drugs, released from the dosage forms can be highly influenced by the colonic transit time. Unlike small intestine, colonic transit time shows considerable variability. Various factors like gender and size of the dosage form, and physiological conditions such as stress, presence of food and disease state influence the colonic transit time. Small particles and solutions pass slowly through proximal colon and in human beings, men show shorter transit time than women. Small variation in dietary fibre and age does not alter the transit time, significantly. The colonic transit time of a capsule in adults is 20-35 h,the transit rate being independent of capsule density and volume. Improved residence time with subsequent longer transit time and the contact of dosage form with micro flora in colon may govern release and absorption of drug from dosage form.

4. Gastric emptying:

Generally, in fasted state, gastric emptying is fastest and most consistent. Emptying completes from 5-10 min up to 2 hours, depending on phase of the stomach at the time of drug administration. Gastric emptying can be considerably slowed by the fed state. For drug delivery to the systemic vasculature, small transit time in stomach to reduce random distribution of particulate drug through intestine is preferable. The residence time in the stomach is important for single unit sustained release systems like tablets, which are designed to deliver drug in large intestine. Such systems may release the drug at a distant locus from the colon. (S P Vyas et al)

5. Stomach and intestinal pH:

Generally, the release and absorption of orally administered drugs are influenced by the gastrointestinal pH. The pH gradient in the GIT is not in an increasing order. In stomach the pH is 1.5-2 and 2-6 in fasted and fed conditions, respectively. The acidic pH is responsible for the degradation of various pH sensitive drugs and enteric coating may prevent it. In small intestine, the pH increases slightly from 6.6-7.5 and decreases to 6.4 in right colon.

The pH of mid colon and left colon is 6.6 and 7.0, respectively. Since there is minimal variation in the pH from ileum to colon, apparently pH dependent polymer drug delivery may not be much selective. However, possible exploitation of pH variation in GIT leads to successful development of various colon specific drug delivery systems.

6. Colonic micro flora and enzymes:

The human alimentary canal is highly populated with bacteria and other micro flora at both ends, that is the oral cavity and the colon or rectum. Microorganisms of the oral cavity, normally do not affect oral drug delivery systems, however, gut micro flora of the colon offers a number of implications in health. The concentration of gut micro florarises considerably high levels in the colon.

The enzyme catalyzed metabolic reactions carried out by the enzymes and secretary products released from the micro flora can be used to deliver drugs selectively to colon. The enzymes which trigger the release of drugs in the colon are glycosidase and glucuronidases.

7. Colonic absorption:

The surface area of the colon is much less compared to small intestine, and hence not ideally suited for absorption. The colon is considered for drug delivery because the environment is devoid of endogenous digestive enzymes other than from microbial origin and the residence time of colon can be as long as 10-24 h. Little mixing in the colon makes it possible to create local environments with optimal absorption conditions. The higher viscosity of colonic content delays the diffusion of drug from the lumen to mucosa. The absorption is influenced by the transport of water, electrolytes and ammonia across the mucosa, and it is more in the proximal colon than the distal colon.

Drug molecules pass from the apical to baso lateral surface of the epithelial cells by

- Passing through colonocytes.
- Passing between adjacent colonocytes.

Absorption enhancers facilitate effective absorption through various mechanisms

- Disruption of the intra cellular occluding junction complex opens the para cellular route.

- Modification of epithelial permeability by denaturing membrane proteins.
- Modification of lipid protein interactions and disruption of the integrity of lipid barrier by colonic enterocytes.

Pharmaceutical Factors:

1. Drug candidates:

Drugs, which show poor absorption from the stomach or intestine including peptide drugs, are most suitable for colon-specific drug delivery systems. The drugs used in the treatment of IBD, ulcerative colitis, diarrhea and colon cancer.

2. Drug carriers:

The selection of carrier for particular drug candidate depends on the physiochemical nature of the drug as well as the disease. The factors such as chemical nature, stability and partition coefficient of the drug and the type of absorption enhancer chosen influence the carrier selection. Moreover, the choice of drug carrier depends on the functional groups of the drug molecule.

CAPECITABINE:

Capecitabine is a pyrimidine analogue used as an antineoplastic agent to treat metastatic and advanced forms of breast and colon cancer. It is an oral pro drug of 5-FU that also is effective in the adjuvant setting and is being evaluated as a replacement for 5-FU for patient convenience and safety reasons. 5-Fluorouracil-based chemotherapy is the standard of care for the adjuvant treatment of colorectal cancer either as a single agent or more commonly, in combination with other agents. Capecitabine was approved for use as an anticancer agent in the United States in 1998 and is currently an important component of several cancer chemotherapeutic regimens. Current indications include advanced, metastatic breast cancer and colorectal cancers, usually after failure of first line therapies. Once swallowed, Capecitabine is rapidly and completely absorbed as an intact molecule through the gastrointestinal mucosa. Capecitabine is not intrinsically cytotoxic, and undergoes a three-step conversion to 5-FU. The first stage is mediated by carboxyl esterase in the liver, and the second step is governed by cytidine deaminase in liver/tumor tissue. The final conversation step, which results in the generation of 5-FU,is being phosphorylation by thymidine phosphorylase (TP). TP levels are

reported to be higher in tumor cells than in normal tissues; therefore, the systemic exposure of active drug is minimized, and tumor concentrations of the active drug are optimized. TP is identical in structure and function to tumor – associated angiogenic factor and platelet- derived endothelial cell growth factor. It includes neovascularization and prevents tumor cells from entering apoptosis. TP expression correlates with fat malignant growth aggressive invasion potential, and poor patient prognosis. TP activation may, therefore, enable capecitabine provides a clear rationale for combining capecitabine with anti-tumor agents that further up regulate TP in tumor tissue. Fluorouracil is further metabolized to two active metabolites, 5-fluoro-2-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP), within normal and tumour cells. FdUMP inhibits DNA synthesis by reducing normal thymidine production, while FUTP inhibits RNA and protein synthesis by competing with uridine tri phosphate. The active moiety of capecitabine, fluorouracil, is cell cycle phase-specific (S- phase).

NANOPARTICLES:

The prefix "nano" comes from the ancient Greek vavoc through the Latin nanus meaning very small. Nanotechnology defined as design characterization, production and applications of structures, devices and systems by controlling shape and size at nanometer scale. According to International System of Units (SI) nanotechnology is typically measured in nanometers scale of 1 billionth of a meter (1nm corresponding to 10-9 m) referred as the "tiny science". At this small size molecules and atoms work differently, behave as a whole unit in terms of its properties and transport, provide a variety of advantages. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. The term nanoparticle is a combined name for both nanosphares and nanocapsules. Drug is confined to a cavity surrounded by a unique polymer membrane called nanocapsules, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Where a conventional technique reaches their limits, nanotechnology provides opportunities for the medical applications. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Advantages in nanoparticle drug delivery, particularly at the systemic level, include longer circulation half-lives, improved pharmacokinetics and reduced side effects. In cancer treatments, nanoparticles can further rely on the enhanced permeability

and retention effect caused by leaky tumor vasculatures for better drug accumulation at the tumor sites.

Nanoparticles were first developed for the parenteral route; more recently, they have also been studied as oral delivery vehicles. Clearly, a wide variety of drugs can be delivered via the oral route using polymeric nano particulate carriers and much of the research has focused on the absorption enhancement of peptides, proteins, and vaccine antigens. Generally conventional preparations like solutions, suspension from certain limitations like higher dose requirement due to low bioavailability which often associated with the first pass effect with intolerance and instability. They are associated with fluctuation in plasma drug levels and do not provide sustained effect. Hence, some novel carriers are need of the hour, which could meet ideal requirement of drug delivery systems. Therefore the nanoparticles are associated with other nano size related property that differs significantly from those observed in fine particles. (Nagavarma B V N et al)

Nanoparticles play an important role in a number of these applications. Nanoparticles which in general terms are defined as engineered structures with diameters of < 100nm are devices and systems produced by chemical and physical processes having specific properties. The reason why nanoparticles are attractive for such purposes is based on their important and unique features, such as their surface to mass ratio, which is much larger than that of other particles and materials, allowing for catalytic promotion of reactions, as well as their ability to adsorb and carry other compounds. The reactivity of the surface originates from quantum phenomena and can make nanoparticles unpredictable since, immediately after generation; nanoparticles may have their surface modified depending on the presence of reactants and adsorbing compounds, which may instantaneously change with changing compounds and thermodynamic conditions. Therefore, on one hand, nanoparticles have a large (functional) surface which is able to bind, adsorb and carry other compounds such as drugs, probes and proteins. On the other hand, nanoparticles have a surface that might be chemically more reactive compared to their fine analogues. The ideal requirement for designing nano particulate delivery system are to effectively control the particle size, surface properties, enhance solubility and release of pharmacologically active ingredients in order to achieve the site specific delivery at

pre-determined rate. Hence the polymeric nanoparticle effects stability of drugs and possess useful control release properties.

Advantages of Nanoparticles:

A nanoparticle offers numerous advantages in drug delivery system. These advantages include, but are not limited:

- Nanoparticles have much significant advantage over conventional and traditional drug delivery system.
- Nanoparticles are control and sustain release form at the site of localization; they alter organ distribution of drug compound. They enhance drug circulation in blood, bioavailability, and therapeutic efficacy and reduce side effects.
- Nanoparticles can be administered by various routes including oral, nasal, parenteral, intra-ocular etc.
- In the tiny areas of body nanoparticles shows better drug delivery as compare to other dosage form and target to a particular cell type or receptor.
- Due to small particle size a nanoparticle overcome resistance by physiological barriers in the body and easily penetrates to cell walls, blood vessels, stomach epithelium and blood–brain barrier.
- Nanoparticle enhances the aqueous solubility of poorly soluble drug, which improves bioavailability of drug.
- As targeted drug carrier nanoparticles reduce drug toxicity and enhance efficient drug distribution.
- By using polymers drug release form nanoparticles can be modified which makes polymeric nanoparticle an ideal drug delivery system for cancer therapy, vaccines, contraceptives and antibiotics.

POLYMERS USED IN THE PREPARATION OF NANOPARTICLES:

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible.

The most commonly used natural polymers in preparation of polymeric nanoparticles are

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

There are many synthetic polymers like

- Poly lactides (PLA)
- Poly glycolides (PGA)
- Poly (lactide co-glycolides) (PLGA)
- Poly anhydrides
- Poly orthoesters
- Poly cyanoacrylates
- Poly caprolactone
- Poly glutamic acid
- Poly malic acid
- Poly (N-vinyl pyrrolidone)

METHODS OF PREPARATION OF NANOPARTICLES:

In the preparation of nanoparticles different types of matrix material are used such as polysaccharides, synthetic polymer and proteins. Various factors are involved in selection of matrix material to be used in preparations which are (Abhishek Garg et al)

(i) Required nanoparticle size.
(ii) Permeability and surface charge of nanoparticle.
(iii) Level of biodegradability and biocompatibility must be optimum.
(iv) Material must not be toxic.

(v) Solubility profile and stability of drug should not be affected.

(vi) It should show desired drug release profile.

(vii) Must not be immunogenic.

Following are methods which are used in formulation of nanoparticles

1. Dispersion of preformed polymers.
2. Polymerization method.
3. Coacervation or ionic gelatin method.
4. Supercritical fluid technology

1. Dispersion of preformed polymers:

Solvent evaporation method:

In this method, there is conventional formation of o/w emulsion between a partially water miscible solvent containing the polymer and the drug, and an aqueous phase containing the stabilizer. In this polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. Oil in water (o/w) emulsion is prepared by emulsification of drug and polymer mixture in aqueous solution which contain emulsifying agent, which result in formation of stable emulsion. After that by using pressure reduction method or continuous stirring, organic solvent is evaporated. The homogenizer speed, nature and stabilizer concentration along with the property of polymer effect size of nanoparticle. Usually high speed homogenizer or ultra-sonication had been used to reduce the size of nanoparticle to an optimum size. Spontaneous emulsification or solvent diffusion method also known as modified version of solvent evaporation method. In this method, two phase solvent is used, one is water miscible and other is water immiscible i.e. organic in nature which act as oil phase. In this method interfacial turbulence is created, by immediate diffusion between two solvents (which are differing in phase) which lead to the formation of small particles. A reduction in particle size can be gained by increasing the concentration of water miscible solvent both the above described method can be used for preparation of hydrophilic and hydrophobic drugs.

Salting out:

It is one of commonly used method used for preparation of nanoparticle. This method involves the mixing of saturated aqueous solution of polyvinyl alcohol (PVA) into an acetone solution of the polymer under magnetic stirring resulting in the formation of o/w emulsion. The precipitation of the polymer occurs when sufficient amount of water is added to external phase to allow complete diffusion of the acetone from internal phase into aqueous phase.

2. Polymerization method:

Polymerization of monomers in an aqueous solution forms the basis of this method. Two different techniques are used for the preparation in aqueous solution.

a) Emulsion polymerization: This method involves emulsification of monomer in non-solvent phase.

b) Dispersion polymerization: This method involves dispersion of monomer in non-solvent phase.

Incorporation of drug in nanoparticle can be achieved either by dissolving the drug in polymerization medium or by adsorption onto nanoparticle. Suspension of nanoparticles is formed, which contain surfactants and stabilizers that are used in polymerization which has to be removed by method like ultracentrifugation or by suspending them in isotonic medium which is free of surfactant. Poly butyl cyanoacrylate or poly (alkyl cyanoacrylate) nanoparticles are been prepared by this method. The polymer particle size had been affected by concentration of stabilizer and surfactant involved in preparation.

3. Production of nanoparticles using supercritical fluid technology:

Various conventional approaches like solvent diffusion, solvent extraction-evaporation and organic phase separation require the use of organic solvent are hazardous to the environment as well as the physiological systems. Supercritical fluid technology thus has been invested as an alternative to prepare biodegradable micro and nanoparticles. Solvent which remain fluid in a single phase regardless of pressure above critical temperature are known as supercritical fluid. Super critical CO_2 is the most widely used supercritical fluid. The most common processing techniques involves supercritical fluids are Supercritical Anti Solvent (SAS) and Rapid Expansion of Critical Solution (RESS).

4. Coacervation or ionic gelation method:

Chitosan nanoparticles were prepared on the basis of the ionic gelation method. At the initial step, defined concentrations of TPP aqueous solution was prepared and added drop by drop to CS solution, which was prepared in 1% acetic acid (v/v); the solution was stirred at 400 - 600 rpm. Different CS/TPP ratios were prepared to determine an appropriate formulation with optimum properties. Particle size distribution of the prepared colloidal suspensions was determined by the light scattering method using a particle size analyzer. Moreover, the effect of sonication on mean particle size was evaluated. (Amir Dustgani et al)

EVALUATION OF NANOPARTICLES:

Drug content:

Accurately weighed powder sample was transferred into a volumetric flask and made up to volume with distilled water. The solution was suitably diluted with distilled water and absorbance was measured using UV-visible spectrophotometer**.**

Entrapment Efficiency:

The procedure of entrapment efficiency was to determine the drug entrapped into the copolymer. The prepared formulations were centrifuged in 2 ml eppendorf tube at 15000 rpm for about 30 minutes. Then the samples were separated into supernatant and precipitated one. The precipitated samples were re dispersed by using buffers and re centrifuged for 3-4 times to get entrapped drug alone. The samples were then subjected for the analysis of UV visible spectrophotometer.

%EE = Total amount of drug - Entrapped drug / Total amount of drug X100

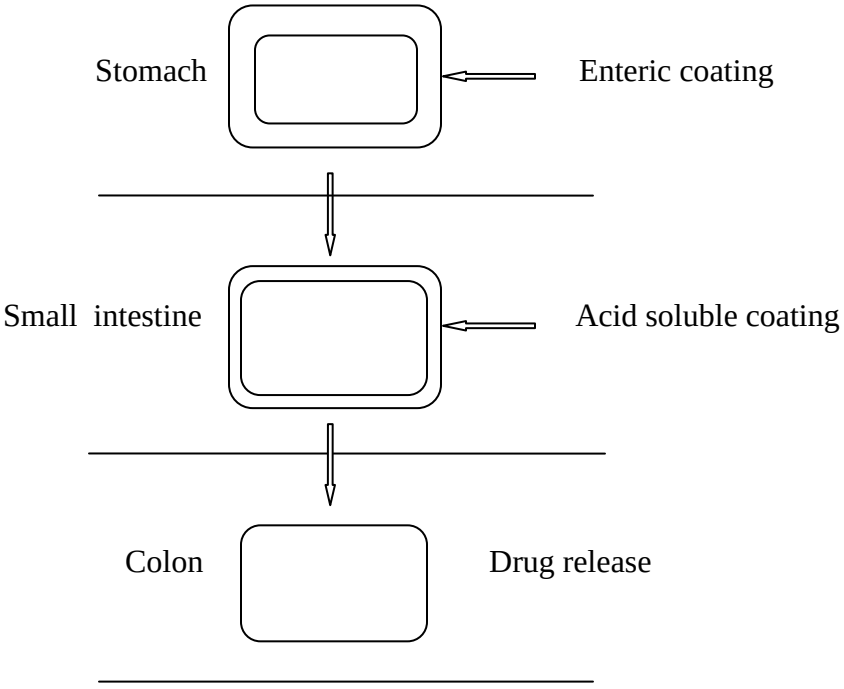
APPROACHES FOR COLON DRUG DELIVERY SYSTEM:

- pH sensitive system
- Time controlled or Time dependent system
- Microbially triggered system
- Targeted prodrug design
- Pressure controlled system
- Osmotically controlled system
- CODESTM

CODESTM:

CODESTM is a combined approach of pH dependent and microbially triggered CDDS. It has been developed by utilizing a unique mechanism involving lactulose, which acts as a trigger for site specific drug release in the colon. The system consists of a traditional tablet core containing lactulose, which is over coated with and acid soluble material, and then subsequently over coated with an enteric material. The premise of the technology is that the enteric coating protects the tablet while it is located in the stomach and then dissolves quickly following gastric emptying. The acid soluble material coating then protects the preparation as it passes through the alkaline pH of the small intestine. Once the tablet arrives in the colon, the bacteria enzymatically degrade the polysaccharide (lactulose) into organic acid. This lowers the pH surrounding the system sufficient to affect the dissolution of the acid soluble coating and subsequent drug release. (Prasanth V. V et al)

Figure 5: MECHANISM OF CODESTM



TABLETS:

Solid dosage forms are the most popular category of pharmaceutical formulations. They are in a sense 'convenience' dosage forms and in comparison to the liquid formulations, their stability is of a high order. Within the solid dosage forms, unitary forms such as tablets, pills, capsules, cachets, wrapped powders rank high since they ensure accuracy in dosage.

A tablet is a pharmaceutical dosage form. Tablets may be defined as the solid unit dosage form of medicament or medicaments with or without suitable excipients and prepared either by molding or by compression. It comprises a mixture of active substances and excipients, usually in powder form, pressed or compacted from a powder into a solid dose. The excipients can include diluents, binders or granulating agents, glidants and lubricants to ensure effective tableting, disintegrants to promote tablet break-up in the digestive tract, sweeteners or flavours to enhance taste and pigments to make the tablets visually attractive or aid in visual identification of an unknown tablet. A polymer coating is often applied to make the tablet smoother and easier

to swallow, to control the release rate of the active ingredient, to make it more resistant to the environment (extending its shelf life) or to enhance the tablet's appearance. (Lieberman et al)

The compressed tablet is the most popular dosage form. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of these are compressed tablets. A tablet can be formulated to deliver an accurate dosage to a specific site; it is usually taken orally, but can be administered sublingually, buccally, rectally or intravaginally. The tablet is just one of the many forms that an oral drug can take such as syrups, elixirs, suspensions and emulsions.

Tablet properties:

- A tablet should have elegant product identity while free of defects like chips, cracks, discoloration, and contamination.
- Should have sufficient strength to withstand mechanical shock during its production packaging, shipping and dispensing.
- Should have the chemical and physical stability to maintain its physical attributes over time.
- Must have a chemical stability over time so as not to follow alteration to the medicinal agents.
- The tablet must be able to release the medicinal agents in a predictable and reproducible manner.

Advantages:

- Greatest dose precision and the least content variability.
- Cost is lowest of all oral dosage form.
- Lighter and compact.
- Easiest and cheapest into package and strip.
- Easy to swallowing with least tendency for hang-up.

Disadvantages:

- Difficult to swallow in case of children and unconscious patients.
- Drugs with poor wetting, slow dissolution properties, may be difficult to formulate or manufacture as a tablet that will still provide adequate or full drug bioavailability.
- Some drugs resist compression into dense compacts, owing to amorphous nature, low density character.
- Bitter testing drugs, drugs with an objectionable odor or drugs that are sensitive to oxygen may require encapsulation or coating. In such cases, capsule may offer the best and lowest cost.

Tablet manufacturing:

The manufacture of granulations for tablet compression may follow one or a combination of three established methods.

1. Direct compression method
2. Dry or compression granulation method
3. Wet granulation method

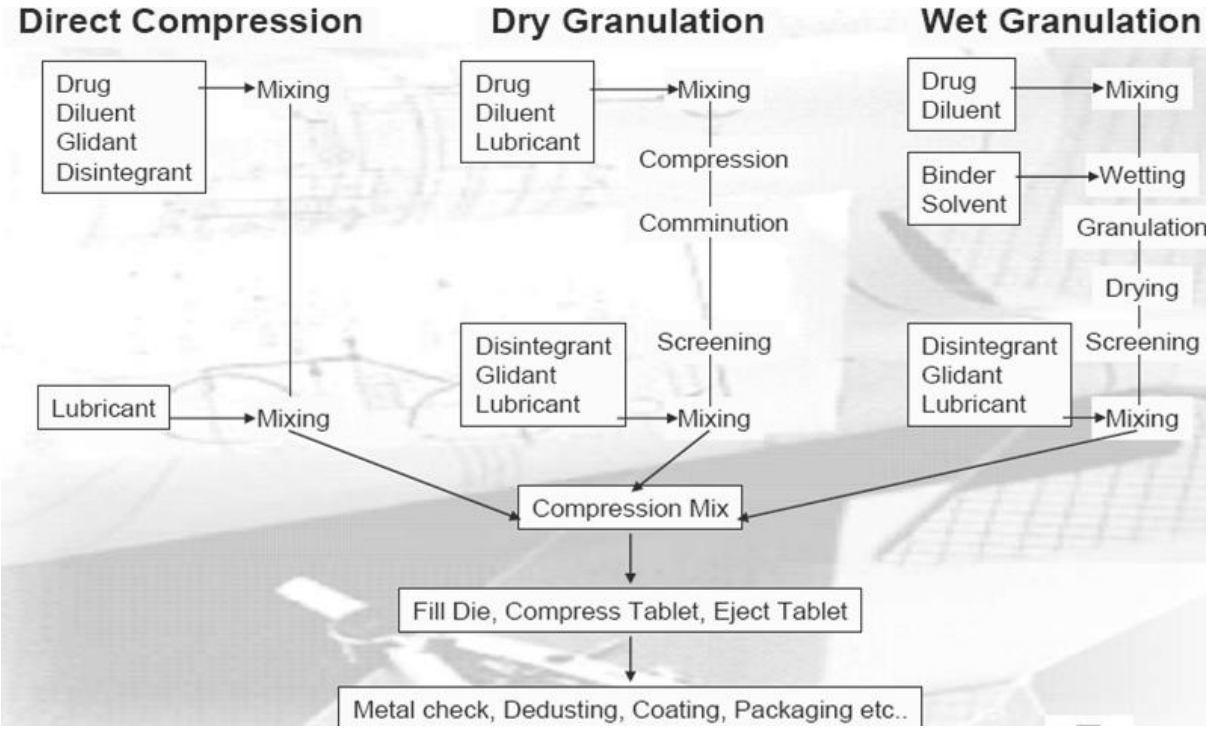
Direct compression method:

Direct compression is a popular choice because it provides the shortest, most effective and least complex way to produce tablets. The manufacturer can blend an API with the excipient and the lubricant, followed by compression, which makes the product easy to process. No additional processing steps are required.

Wet granulation method:

Granules are formed by the addition of a granulation liquid onto a powder bed which is under the influence of an impeller. The agitation resulting in the system along with the wetting of the components within the formulation results in the aggregation of the primary powder particles to produce wet granules. The granulation liquid contains a solvent which must be volatile. So that it can be removed by drying, water, ethanol, isopropanol are used. The liquid solution can be either aqueous based or solvent based.

Figure 6: METHODS OF COMPRESSION



Dry granulation method:

It is used to form granules without using a liquid solution because the product granulated may be sensitive to moisture and heat. Forming granules without moisture requires compacting and densifying the powders. In this process the primary powder particles are aggregated under the high pressure. When a tablet press is used for dry granulation, the powders may not possess enough natural flow to feed the product uniformly into die cavity.

DELAYED RELEASE DOSAGE FORMS:

A delayed release dosage form is designed to release the drug at a time other than promptly after administration. Dosage forms can be designed to modify the release of the drug over a given time or after the dosage form reaches the required location. Delayed release oral dosage forms can control where the drug is released, e.g, when the dosage form reaches the small intestine (enteric coated dosage forms) or the colon (colon-specific dosage forms). Delayed release systems release a bolus of the drug after a predetermined time in a predetermined location, i.e. they do not release the drug immediately after ingestion, for example enteric coated tablets, pulsatile-release capsules.

The coatings that are used today to produce enteric effects are primarily mixed acid functionality and acid ester functionality synthetic or modified natural polymers. Cellulose acetate phthalate has the longest history of use as an enteric coating. More recently, polyvinyl acetate phthalate and hydroxyl propyl methyl cellulose phthalate have come into use. All three polymers have the common feature of containing the di carboxylic acid, phthalic acid, in partially esterifies form. These polymers, being acid esters, are insoluble in gastric media that have a pH of up to about 4; they are intended to hydrate and begin dissolving as the tablets leave the stomach, enter the duodenum (pH of 4 to 6), and move further along the small intestine, where the pH increases to a range of 7 to 8. The primary mechanism by which these polymers lose their film integrity, thereby admitting intestinal fluid and releasing drug, is ionization of the residual carboxyl groups on the chain and subsequent hydration. The presence of esterases in the intestinal fluid that break down ester linkages of the polymer chains may also play some role, as may surface activity effects of bile salts and other components in bile that enter the upper small intestine via the bile duct.

Delayed release dosage forms are designed to provide spatial placement or temporal targeted delivery of a drug to the distal human gut. Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to desired rate of drug release to target tissue over a specified period of time. The primary aim of using delayed release products is to protect the drug from gastric fluids, to reduce gastric distress caused by drugs particularly irritating to the stomach or to facilitate gastrointestinal transit for drugs that are better absorbed from intestine. Delayed release products are typically enteric-coated or targeted to the colon.

EVALUATION OF TABLETS:

PRE COMPRESSION PARAMETERS:

Angle of repose:

The manner in which stresses are transmitted through a bead and the beads response to applied stress are reflected in the various angles of friction and response. The most commonly used of this in angle of repose, which may be determined experimentally by number of methods. The method used to find the angle of repose is to pour the powder a conical on a level, flat surface and measure the included angle with the horizontal.

$$\theta = \text{Tan}^{-1} (h/r)$$

Where, θ = Angle of repose, h = Height of the powder cone, r = Radius of the powder cone.

Bulk density:

Bulk density of a compound various substantially with the method of crystallization, milling or formulation. Bulk density is determined by pouring pre sieved granules into a graduated cylinder via a large funnel and measure the volume and weight. (Pavani G et al)

Bulk density = weight of granules ÷ Bulk volume of granules

Tapped density:

Tapped density is determined by placing a graduated cylinder containing a known mass of granules and mechanical tapper apparatus, which is operated for a fixed number of taps until the powder bed volume has reached a minimum volume, using the weight of the drug in the cylinder and this minimum volume, the tapped density may be computed.

Tapped density = weight of granules÷ Tapped volume of granules

Carr's index:

Carr's index is measured using the values of bulk density and tapped density. The following equation is used to find the Carr's index

$$CI = (TD-BD) \times l \div \text{Tapped density}$$

Where TD = Tapped density

BD = Bulk density

Hausner's Ratio:

It indicates the flow properties of the powder and ratio of Tapped density to the Bulk density of the powder or granules.

Hausner's Ratio = Tapped density ÷ Bulk density

POST COMPRESSION PARAMETERS:

Physical appearance:

The physical appearance of the compressed tablets involves the measurement of a number of attributes like tablet shape, smoothness, chipping, cracks, surface texture, color, embossing.

Thickness:

Thickness was determined for 20 pre weighed tablets of each batch using a digital vernier scale and the average thickness was determined in mm. The tablets thickness should be controlled within a ±5% variation of standard.

Weight variation:

20 tablets were selected randomly from a batch and were weighed individually and then average weight was calculated. The tablets meet the USP specifications if not more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.

Hardness test:

The crushing load which is the force required to break the tablet in the radial direction was measured using hardness tester. The hardness of 10 tablets was noted and the average hardness was calculated.

Friability test:

In friability testing the tablets are subjected to abrasion and shock. It gives an indication of the tablets ability to resist chipping and abrasion during transportation and shipping. Tablets initial weight was noted. The tablets were rotated in Roche Friabilator for 100 revolutions at 25rpm. The tablets were dedusted and reweighed. The %friability should be not more than l% w/w of the tablets being tested. The % friability is expressed as the loss of weight and is calculated

$$\% \text{ Friability} = (W_o - W_f) / W_o \times 100$$

Wo - initial weight of tablets

W_f -final weight of tablets

Disintegration time:

It is the time taken by the tablet to breakup into smaller particles. The disintegration test is carried out in an apparatus containing a basket rack assembly with six glass tubes of 7.75 cm in length and 2.15mm in diameter, the bottom of which consists of a 10 mesh sive. The basket is raised and lowered 28 - 32 times per minute in a medium of 900ml which is maintained at 37˚C. Six tablets were placed in each of the tubes and the time required per complete passage of tablet through the 10 mesh was considered as the disintegration time of the tablet.

Dissolution studies:

Dissolution is process by which a solid state enters a solution. In pharmaceutical industry it may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid (or) solid interface, temperature and solvent composition. Dissolution is considered as one of the most important QC test performed on pharmaceutical dosage form and is now developing into a tool for predicting bioavailability

A) Apparatus1 (Basket Type): A single tablet is placed in a Wire mesh basket attached to the bottom of the shaft connected to a variable speed motor, the basket is immersed in a dissolution medium contained in a 1000 ml flask. The flask is cylindrical with hemispherical bottom and maintained at 37oC ± 0.5oC by a constant temperature bath. The motor is adjusted to turn at the specified speed and sample of the fluid is withdrawn at intervals' to determine the amount of drug in solution.

B) Apparatus2 (paddle type): It is same as apparatus 1 except basket is replaced by paddle. The dosage form is allowed to sink to the bottom of the flask before stirring. For dissolution test USP specifies the dissolution test medium and volume, type of apparatus to be used, RPM of the shaft, the time limit of the test, and assay procedure. The test tolerance is expressed as a % of the labeled amount of drug dissolved in the limit.

LITERATURE REVIEW

Amir Dustgani et al., (2008) have studied about chitosan nanoparticles were used to describe the synthesis and characterization of novel biodegradable nanoparticles for encapsulation of dexamethasone sodium phosphate. Ionic gelation method was used for preparation of nanoparticles. These drug containing nanoparticles were prepared with different amounts of drug. The method based on the interaction between the negative groups of sodium tri polyphosphate (TPP) and the positively charged amino groups of chitosan. Dexamethasone sodium phosphate loaded nanoparticles were obtained according to the same procedure. The mean size and size distribution of nanoparticles were measured by dynamic laser light scattering.

S. Latha et al., (2012) have formulate the capecitabine loaded nanoparticles of chitosan (CS) cross linked with tri polyphosphate (TPP) for anti-cancer therapy, in order to enhance the bioavailability and reduce dose frequency. Then the nanoparticle solution was prepared by dissolving chitosan (CS) in 1% (w/v) acetic acid solution under magnetic stirring under room temperature. The chitosan solution was diluted with deionized water to produce different concentration. The nanoparticle average size was found to be in the range of 120-250 nm and it is spherical in shape with high zeta potentials. *In vitro* release studies in phosphate buffer pH 7.4 showed an initial burst effect and followed by a slow drug release. Then the drug release followed zero order kinetics. This study concluded that the encapsulation of capecitabine into chitosan can significantly improve the anticancer activity.

Varshosaz J et al., (2011) have discussed about the development of budenoside pellets based on a novel colon drug delivery system. Budenoside is the drug of choice for treatment of active inflammatory bowel disease (IBD). Pellets were prepared by extrusion (or) spheronization and coated with an acid soluble polymer, HPMC and an enteric coated polymer sequentially. *In vitro* drug release of coated pellets was studied using USP dissolution apparatus type 2 in buffers of pH 1.2,7.4,6.8.The morphology of optimized formulations of coated pellets was characterized using scamming electron microscopy. This study suggests that pellets based on codestm technology could be useful for colonic delivery.

Kishore M et al., (2015) explained that a novel colon specific drug delivery system of an Anti hypertensive drug is atenolol, for treatment of chronic cardiac diseases like Heart failure, sudden

increases in blood pressure was developed. Mini tablets of Atenolol were prepared by wet granulation method using matrix forming natural polymers. The further effect of enteric coat on the mini tablets for colon specific drug release was investigated. The Atenolol optimized matrix formulation shows drug release around 32.37±0.33% in 2 hrs. So it was further enteric coated with eudragit S100 in cumulative ratio and formulated the formulations. Apart from the formulation showed optimum drug release after 24 hrs. All formulations were subjected to Hardness test, Friability test, determination of uniform diameter and thickness, drug content for optimization and further evaluation. In vitro dissolution studies revealed that the drug release in upper part of GIT from matrix tablets of Atenolol can be prevented by enteric coating with pH sensitive polymer (EudragitS100), which releases the drug specifically in colonic region to achieve target delivery. For drug release three dissolution media with pH 1.2, 7.4 and 6.8 were used sequentially. These three media represents the stomach, proximal part of the small intestine and terminal ileum respectively.

Claudia S. LEOPOLD et al., (1998) have described about the study was to investigate an acid-soluble polymer as coating material for multiple units with regard to its ability to allow drug release only under the acidic conditions of the inflamed colon. Mini tablets containing 20% (w/w) of the model drug dexamethasone with or without the muco adhesive swelling agent carbomer 934 (neutralized) were coated in a small coating pan with different amounts of an organic solution of eudragit E. Drug release from the eudragit E-coated cores at pH 2.0- 5.0 starts after 10-50min due to the rapid dissolution of the eudragit E film. At pH 6.8 lag times of drug release depend on the composition of the cores and the thickness of the coating film. In the case of the carbomer containing cores drug release is induced by disruption of the coating film due to swelling of the cores and lag times (up to 20 h) increase over proportionately with increasing coating thickness. With no swelling agent in the cores drug release at pH 6.8 is delayed due to the low erosion (or) dissolution rate of eudragit E. Lag times of drug release (up to 33 h) increase in a linear manner with increasing coating thickness. Thus, eudragit E, protected against dissolution in the stomach by an enteric coating, is a suitable coating polymer for drug release in acidic regions such as the inflamed colon.

Sampath et al., (2014) stated that long-term uses of non-selective NSAIDs can lead to gastrointestinal toxicity from sustained inhibition COX-1. But one can overcome such problem

by formulating them as colon specific delivery. The present study was carried out to develop oral colon targeted drug delivery system for nimesulide utilizing recently designed and patented system called CODESTM, which consisted of a lactulose containing core over coated with both eudragit E and eudragit L designed to rapidly disintegrate in the colon, in order to give a new life for an existing banned drug. CODESTM tablets were prepared by tableting the granulation of nimesulide and lactulose, followed with film coating. The prepared tablets were evaluated on the basis of various pharmacopoeial characteristics. The onset of nimesulide release was found to dependent on the coating level of eudragit E, and at eudragit E, the onset of in vitro drug release was found to be optimum. It is concluded that nimesulide can be targeted to hindgut by Novel approach of CODESTM in a simple and economic way**.** From the obtained results, it can be concluded that drug release and absorption of nimesulide can be targeted on the colon by CODES system. Evaluation parameters like hardness and friability indicated that the tablets so prepared were mechanically stable and complied with necessary pharmacopoeial specifications. I.R. spectra revealed that, the drug and polymers are authentic and match with the reference standard available and there is no interaction between polymers and drug.

Sukhbir Kaur et al., (2014) have investigated about matrix tablets of indomethacin were prepared by wet granulation method. Guar gum and Pectin as a carrier, 10% starch paste, di calcium phosphate is used as diluents and the mixture of talc and magnesium stearate at 2:1 ratio were used. All the prepared formulations were evaluated for hardness, drug content uniformity and were subjected to in vitro drug release studies. *In vitro* drug release study was conducted at 37 ºC and 100 rpm for 2 h in 900 ml buffer of pH 1.2. The dissolution medium were replaced with 900 ml of pH 7.4 phosphate buffers and tested for drug release up to 3 h. Drug release study was continued for 24 hours in 6.8 pH phosphate buffer. Colorectal cancer, is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix. It is forth most commonly diagnosed cancer in the world. Symptoms of the disease include rectal bleeding and anemia which are sometimes associated with weight loss, changes in bowel habits, fever, loss of appetite, and nausea or vomiting. So in order to treat this disease colon targeted delivery of indomethacin is designed using natural polysaccharides i.e. pectin and guar gum for site specific delivery. These carriers were degraded by colonic bacteria hence delivering the drug colon region.

Jennifer I. Hare et al., (2013) have investigated the use liposomal irinotecan (Irinophore C^{TM}) plus or minus 5-fluorouracil (5-FU) for the treatment of colorectal cancer. The effect of irinotecan (IRI) and/or 5-FU exposure times on cytotoxicity was assessed *in vitro* against HT-29 or LS174T human colon carcinoma cells. The pharmacokinetics and bio distribution of Irinophore C^{TM} (IrC^{TM}) and 5-FU, administered alone or in combination, were compared in vivo. A subcutaneous model of HT-29 human colorectal cancer in Rag2-M mice was utilized to assess the efficacy of IrC^{TM} alone, and in combination with 5-FU. This strategy has been shown to be important for producing synergistic anti-cancer effects with floxuridine and other drug combinations. Finally, it may be prudent to explore the use of leucovorin to potentiate the efficacy of 5-FU in the HT-29 model.

Subal Chandra Basak et al., (2008) studied about a metformin hydrochloride (metformin HCl) was formulated as a hydrophobic matrix sustained release tablet employing wax materials and the sustained release behavior of the fabricated tablet was investigated. The tablets were prepared by wet granulation technique. The formulation was optimized on the basis of acceptable tablet properties and in vitro drug release. Then the resulting formulation produced monolithic tablets with optimum hardness, uniform thickness, consistent weight uniformity and low friability. Statistically significant differences were found among the drug release profile from different bees wax combination matrices. The results of dissolution studies exhibited drug release pattern very close to theoretical release profile. Applying kinetic equation models, the mechanism of release of the drug from the three formulations was found to be followed Higuchi model, as the plots showed high linearity, with correlation coefficient (R^2) value of 0.98 or more. Tablet matrices containing cetyl alcohol gave better release of the drug than other materials studied. However, the rate of release varied with amount of cetyl alcohol in the matrix. The ‘n’ value lies below 0.5 demonstrating that the mechanism controlling the drug release was the quasi Fickian. Approach of the study was to make an evaluation of wax materials as sustained release matrix for water soluble drug, metformin HCl and to assess the kinetics of drug release mechanism. Bees wax and cetyl alcohol can be used for sustained release of metformin HCl. The study reveals that, the release of water soluble drug, metformin HCl exhibited diffusion dominated mechanism. The wax and cetyl alcohol ratio plays an important role in overall release of the drug. The hydrophobic wax matrix tablet is a promising approach to achieve appropriate sustained release dosage.

Ghassan Zuhair Abdullah et al., (2013) have explained about micro emulsions and nano emulsions are well known candidates to deliver drugs locally. However, the poor rheological properties are marginally affecting their acceptance pharmaceutically. This work aimed to modify the poor flow properties of a nano scaled emulsion comprising palm olein esters as the oil phase and ibuprofen as the active ingredient for topical delivery. Three carbopol resins: 934, 940 and ultrez 10, were utilized in various concentrations to achieve these goals. Moreover, phosphate buffer and tri ethanolamine solutions pH 7.4 were used as neutralizing agents to assess their effects on the gel-forming and swelling properties of carbopol 940. The addition of these polymers caused the produced nano scaled emulsion to show a dramatic droplets enlargement of the dispersed globules, increased intrinsic viscosity, consistent zeta potential and transparent-to-opaque change in appearance. Carbopol 940 and tri ethanolamine appeared to be superior in achieving the proposed tasks compared to other materials. The higher the pH of tri ethanolamine solution, the stronger the flow modifying properties of carbopol 940. Transmission electron microscopy confirmed the formation of a well arranged gel network of carbopol 940, which was the major cause for all realized changes. This study resulted in in vitro and in vivo pharmacodynamics properties that are comparably higher than the reference chosen for this study.

Sovan Lal Pal et al., (2011) describes the development of novel drug delivery systems using nanoparticles. Nanoparticles can offer significant advantages over the conventional drug delivery in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different route of administration and the capability to deliver both hydrophilic and hydrophobic drug molecules. This review focuses on classification, methods of preparation, characterization, application, advantages of nanoparticles and health perspectives.

Pranav Palshikar et al., (2013) this study was to develop an enteric coated tablet of sodium valproate using cellulose acetate phthalate as enteric coating material. Core tablet were prepared by non-aqueous granulation method and seal coated with PVP K-30 which act as moisture barrier. This seal coated tablet was further coated with cellulose acetate phthalate to dissolve in the intestinal fluid. The *in vitro* release result showed that enteric coated were capable of restricting release in the acidic media. The enteric coating was applied with the consideration of

transit time of food or dosage form from stomach to jejunum of small intestine and from percent release verses time plot was plotted.

Sateesh Kumar Vemula et al., (2015) the aim of the study is focused on developing flurbiprofen colon-specific tablets based on timed release and pH-sensitivity. The effect of eudragit coating on the drug release from hydroxyl propyl methylcellulose matrix to achieve the colon-specific release. Tablets were prepared by wet granulation method and coated with eudragit S100 using dip coating method. Then the tablets were evaluated for different physical parameters, compatibility studies, in vitro dissolution and in vivo x-ray imaging studies. Flurbiprofen colon-specific tablets have been characterized for weight variation, hardness, friability and drug content. Compatibility studies revealed that there was no interaction between drug and the polymers. In support of the dissolution studies, x-ray imaging studies revealed that tablets reached the colon without disintegrating in the upper gastro intestinal tract. It concluded that the developed flurbiprofen enteric coated matrix tablets are suitable for colonic delivery.

Hina Kouser Shaikh et al., (2015) this study reviewed the application of mathematical models for study of release phenomenon of drug from dosage forms. Mathematically, it is easy to identify the designing of a particular pharmaceutical system and it can be used to predict the effect of device design parameters on the release kinetic of the formulation. It is easier to obtain the quantitative analysis of data for dissolution release rate by using these models. The choice of selection of best model depends on the desired or required predictive ability and accuracy of the model. This review gives the idea about the current state of mathematical modeling of drug delivery including empirical (or) semi empirical and mechanistic release models. The drug release shows the relationship between drug dissolution and geometry on drug release patterns mathematically. The fact that drug delivery system with multi layered tablet has shown promising results in drug delivery technology and ease of manufacturing is an advantage to the pharmaceutical industries.

V R Sinha et al., (2006) have described to formulate fast release enteric-coated tablets for drug delivery to the colon. Two different approaches were used for the preparation of these tablets. The first included making use of super disintegrant (SD) in the tablet. The amount of super disintegrant in the tablet and the coat weight were varied to formulate a suitable time-controlled release system that would provide colon-specific drug delivery. The second approach consisted

of development of osmogen-based tablets for drug delivery into the tracts of the colon. Two different osmogens, sodium chloride and potassium chloride, were used. These also were coated at different coat levels. Celecoxib was used as a model drug. *Invitro* drug release studies showed that super disintegrants were more effective in showing burst effect in the tablets and therefore showed a rapid drug release as compared with osmogens, which would show a sustained drug release all through the colon. Osmotic tablets were formulated making use of a high concentration of osmogen sodium chloride and potassium chloride were further enteric coated.

Namrata Patel et al., (2015) have stated about capecitabine beads were formulated by an ionotropic gelation method. Analysis of response surface plots allowed identification of an optimized formulation with high drug entrapment and controlled release. Insignificant differences in observed and predicted values for responses validated the optimization method. Optimized beads possessed an average diameter and good flow properties. Their production as spherical beads having a smooth surface was confirmed by scanning electron microscopy. Fourier transform infrared spectroscopy revealed the compatibility of drug with added excipients while differential scanning calorimetry study confirmed the complete drug entrapment in polymer matrix. Higher swelling of beads in phosphate buffer pH 7.4 was obtained in comparison to pH 6.8. *Invitro* dissolution studies of beads loaded into enteric coated capsules revealed negligible release in simulated gastric as well as intestinal fluid, followed by 49.23% release in simulated colonic fluid, in 4 hours. In conclusion, the formulated beads showed colon specific controlled release properties and thus could prove to be effective for colon cancer treatment.

M. P. Bhandarwad et al., (2015) explained about pH dependent system and time dependent system, microbial degradation at the required site is effective for treatment. Solvent less compression coating is one of the strategies for delivering drugs to the colon based on microbial degradation. The aim of the research was to develop a polymer based compression coated tablet of 5-Fluorouracil and to identify the most suitable polymer either alone or in combinations for colonic delivery. Core tablets of 5-Fluorouracil were compression coated with various proportions of Guar gum, xanthum gum and Chitosan. Drug release studies were performed in simulated gastric fluid (SIG) for 2 hours followed by simulated intestinal fluid (SIF) up to 24 hours, demonstrating that the rate of drug release is dependent upon the nature and

concentration of polymer and the pH of environment. The coat containing guar gum or xanthum gum alone showed 30-40% drug release in 8 hours in SIF while guar gum with xanthum gum showed 30-35% drug release in 8 hours in SIF. The coat containing all three polymers showed 45 to 50% drug release in SIF. Further, in vitro dissolution studies performed in the dissolution media with 2% rat ceacal content showed significantly increased drug release because of microbial polysaccharidase enzyme**.**

Nandgude Tanagi Dilip et al., (2016) have briefly explained about to develop a multi particulate system containing chitosan microspheres for colon-specific drug delivery of capecitabine for the treatment of colorectal cancer. This study was based on the microbial degradability of chitosan microspheres. The microspheres were prepared with chitosan by emulsion cross linking method. The effect of concentration of chitosan and drug: Polymer ratio was studied on particle size, % entrapment efficiency, and % drug release. The prepared microspheres also analyzed for percentage yield, flow properties, and surface morphology. The results of analysis of variance test for responses measured indicated that the test is statistically significant. *In vitro* drug release studies were performed in a pH progression medium mimicking the conditions of the gastro intestinal tract showed a fast drug release initially demanded micro encapsulation.

Laila Fatima Ali Asghar et al., (2009) the primary objective of the study was to develop a pH and transit time controlled sigmoidal release polymeric matrix for colon-specific delivery of indomethacin. Tablet matrices were prepared using a combination of hydrophilic polymers (polycarbophil or carbopol) having pH sensitive swelling properties with hydrophobic polymer ethyl cellulose. The prepared matrices were characterized for physical properties and in vitro release kinetics. The presence of ethyl cellulose in a hydrophilic polymer matrix resulted in a sigmoidal in vitro drug release pattern with negligible to very low drug release in the initial phase (0–6 h) followed by controlled release for 14–16 h. The retardation in initial release can be attributed to the presence of ethyl cellulose that reduced swelling of hydrophilic polymer while in the later portion, polymer relaxation at alkaline pH due to the ionization of acrylic acid units on carbopol and polycarbophil resulted in enhanced drug release. Thus, a sigmoidal release pattern was obtained that could be ideal for colonic delivery of indomethacin in the potential treatment of colon cancer.

Jitendra Jagtap et al., (2013) have discussed about the delivery of drugs to the colon through oral route is valuable in treating diseases of the colon with the expectation to protect the drug during the transit time in the gastrointestinal tract and to allow its release only in the colon. This study was to develop colon targeted drug delivery system for satranidazole that is used in the treatment of amoebiasis. Matrix tablets containing a combination of guar gum and hydroxyl propyl methyl cellulose (HPMC) K4M in different ratios were prepared by wet granulation technique followed by enteric coating with eudragit S100. Citric acid was also added, which might further facilitate drug dissolution and absorption. All formulations were evaluated for hardness, swelling, drug content and in-vitro drug release studies. The results of the studies showed that colon targeted matrix tablet of satranidazole containing guar gum and HPMC K4M in the ratio proportion of 3:1 does not released drug in 0.1N HCl (pH 1.2) and small intestine (phosphate buffer, pH 7.4). When the dissolution study was continued in colonic fluids (phosphate buffer, pH 6.8. It was expected that guar gum could be degraded by colonic micro flora containing anaerobic microorganism and the release may be controlled by HPMC K4M and citric acid. Studies demonstrated that orally administered satranidazole matrix tablets can be used effectively for the delivery of the drug to the colon.

Sahdeo Prasad et al., (2012) stated about the development of chemo resistance, poor prognosis, and metastasis often renders the current treatments for colorectal cancer (CRC) ineffective. Whether ursolic acid (UA), a component of numerous medicinal plants, either alone or in combination with capecitabine, can inhibit the growth and metastasis of human CRC was investigated. The effect of UA on proliferation of colorectal cancer cell lines was examined by mitochondrial dye-uptake assay, apoptosis by esterase staining, DNA binding assay and protein expression by western blot. This study resulted that UA inhibited the proliferation of different colon cancer cell lines. Overall our results demonstrate that UA can inhibit the growth and metastasis of CRC and further enhance the therapeutic effects of capecitabine through suppression of multiple biomarkers linked to inflammation, proliferation, invasion, angiogenesis, and metastasis.

Sylwia Flis et al., (2009) have explained about COX inhibitors appear to be promising agents in combination with cytostatics in the treatment of colorectal carcinoma (CRC). The aim of this study was to compare growth inhibitory effects of cytostatics (5-fluorouracil, 5-FU; oxaliplatin)

and COX inhibitor sulindac sulfide (an active metabolite of sulindac), given alone or in combination, on several CRC cell lines. A series of human CRC cell lines were incubated with various combinations of the test drugs used in concentrations from 3 to 200 μm. The cell survival was assessed by MTT assay. Cell cycle progression and apoptosis were measured using flow cytometric methods. In addition, growth inhibitory effects of studied agents on CRC cell lines were compared with a normal cell line. Sulindac sulfide synergistically potentiated the inhibitory effects of 5-FU and oxaliplatin on CRC survival, parallel to the induction of apoptosis. A dose reduction effect for synergistic activity of sulindac sulfide with studied cytostatics suggested that the inhibitory effect of cytostatics on CRC survival may be obtained at low doses. It was apparent that combination of 5-FU or oxaliplatin with sulindac sulfide results in a powerful inhibition of growth of colorectal carcinoma cells in vitro, which may be more specific for cancer than normal cells. The effect of combinations of sulindac sulfide with 5-FU or oxaliplatin on survival of human CRC cells of various cell lines was studied *in vitro.*

Prasanth V.V et al., (2012) stated about colon specific drug delivery system in order to develop drug delivery systems that are able to release drugs specifically in the colon in a predictable and reproducible manner. The colon is a site where both local and systemic delivery of drugs can take place. To achieve successful colon targeted drug delivery, a drug need to be protected from degradation, release and absorption in the upper portion of the gastric intestinal tract (GIT) and then to be ensured abrupt or controlled release in the proximal colon. This review is aimed at understanding recent approaches for dosage forms which is targeting to colon through pH sensitive system, microbially triggered system i.e., pro drugs and polysaccharide based system, timed release system, osmotically controlled drug system, pressure dependent release system. It concluded that the colonic region of the GIT has become an increasingly important site for drug delivery and absorption. Drug targeting to the diseased colon is advantageous in reducing the systemic side effects, lowering dose of a drug, supply of the drug only when it is required and maintenance of the drug in its intact form as close as possible to the target site. All the approaches of colon drug delivery provide means for treatment of local diseases associated with the colon or for systemic absorption of poorly absorbable drugs.

A Maria John Newton et al., (2012) the study was designed to evaluate the in vitro dissolution characteristics of pH-sensitive polymer (HPMC E 15 LV) coated tablets in various simulated

fluids. The mesalamine tablets were fabricated by mixing the drug with micro crystalline cellulose and other ingredients. The fabricated mesalamine tablets were coated with eudragit L100 polymer and HPMC E 15 LV. The fluctuation in colonic pH conditions during inflammatory bowel disease and the nature of less fluid content in the colon may limit the expected drug release in the colon. The different batches of mesalamine tablets were coated with increasing concentration of eudragit L100 and HPMC E 15 LV. The different buffer conditions were chosen to mimic the pH changes in the terminal part of the ileum as well as in the colon. The drug release profile was analyzed for colon-targeting performance in vitro. The kinetics of the drug release also evaluated the release pattern that was best fitted with Higuchian release. The results of the mechanism of release revealed that drug release was found to be a complex one with diffusion, erosion and swelling. Mesalamine delayed release tablets was prepared successfully by coating with eudragit L100 and HPMC E15 LV as polymers to retard the drug release and achieve a required dissolution profile. The drug release studies revealed that the pH-sensitive eudragit L100 can retard the drug release from the tablet until the tablet reaches the colon. The coated tablets released maximum amount of drug in pH conditions of the colon and the terminal part of the intestine. Drug release kinetics indicated that drug release was best fitted to the Hixson–Crowell release kinetics. The "n" value of Korsmeyer–Peppas confirmed that the complex mechanism of swelling diffusion and erosion was involved in the drug release. This study suggests that this simple coating technique may be considered as an alternate method for delivery of drug to the colon to achieve better targeting and pharmacological effects in the treatment of IBD.

G. Pavani et al., (2013) the objective of the present investigation was to formulate and devaluate sustained release of capecitabine tablets. capecitabine sustained release tablets were developed different polymers HPMC K 100, carbopol 974 and Xanthan Gum with different ratios. Sustained release tablets of capecitabine were prepared by wet granulation technique. The prepared granules evaluated in terms of their pre compression studies like tapped density, bulk density, angle of repose, carr's Index and hausner's ratio. The tablets were evaluated by post compression studies like hardness, thickness, friability and in vitro studies. From the dissolution study the R^2 value of Higuchi model is very near to 1 than the R^2 values of the other kinetic models.

Tarak J Mehta et al., (2011) the aim of present study was to develop colon-specific drug delivery systems based on polysaccharide chitosan, were evaluated using in-vitro method. Metronidazole is choice of drug for intestinal amoebiasis. These drugs are to be delivered to the colon for their effective action against E. histolytica wherein the trophozoites reside in the lumen of the caecum and large intestine and adhere to the colonic mucus and epithelial layers. But the pharmacokinetic profile of metronidazole indicates that the drug is completely and promptly absorbed after oral administration. The administration of this drug in conventional tablet dosage form provides minimal amount of metronidazole for local action in the colon, still resulting in the relief of amoebiasis, but with unwanted systemic effects. The Amount of chitosan and carbopol 934P showed significant effect on the release of metronidazole from the colon specific tablet formulation. Present study summarized that chitosan and carbopol can be used successfully to deliver the drug in to colon.

Venkateswara Reddy et al., (2015) have discussed about the sustained release matrix formulation of meloxicam targeted to colon by using various polymers developed. Meloxicam is a selective cyclooxygenase-2 inhibitor with pH-dependent solubility. To achieve pH-independent drug release of meloxicam, pH modifying agents (buffering agents) were used. Colon targeted tablets were prepared in two steps. Initially core tablets were prepared and then the tablets were coated by using different pH dependent polymers. Ethyl cellulose, eudragit L100 and S100 were used as enteric coating polymers. The pre compression blend of all formulations was subjected to various flow property tests and all the formulations were passed the tests. The tablets were coated by using polymers and the coated tablets were subjected to various evaluation techniques. The prepared formulations which may be used for prolong drug release in colon for, thereby improving patient compliance and bioavailability.

Teelavath Vijayakumari et al.,(2015) stated about to develop colon targeted matrix tablets of glipizide using various concentrations of selected polymers are hydroxy propyl methyl cellulose and Guar gum. Tablets were prepared by direct compression method. The compatibility of drug, polymers and excipients were studied by FT-IR spectroscopy. Dissolution studies were performed for 12 hours in 1.2 pH, 7.4 pH, 6.8 pH respectively in phosphate buffer at the temperature of 37±0.5˚C at 100 rpm. The dissolution data so obtained was fitted to various mathematical kinetic models and the drug release followed mixed order and Higuchi's model. To

study release mechanism of the drug from matrices the data were fitted to Koresmeyer – peppas model. In vitro release profile of glipizide from all polymers which are used in study showed that drug increasing the concentration of polymers resulted in a reduction in the release rate of the drug.

Anbarasan B et al., (2015) this present study was to investigate the synthesis of Fe_3O_4 nanoparticles by chemical precipitation method. The Fe_3O_4 nanoparticles were coated with the polymers PLGA- PEG and loaded with the drug capecitabine for the targeting of colon cancer which will be distributed in the large intestine by applying, the external magnetic field. It gets localized in the area of colon cancer cells. After the applied external magnetic field, the iron oxides get heated to 37ºC - 40ºC and the tumour cell gets destroyed. They were very smart materials and mostly used for the applications in medicine like targeted drug delivery system, diagnostic cancer imaging and their therapeutic applications. Also the Entrapment Efficiency (%EE) of the drug was calculated. Further, the particle size can be found by particle size analyzer (PSA). The surface morphology of the formulations was analyzed by SEM analysis.

Vijaya Muthumanikandar et al., (2011) explained about the obtained pure drug mesalamine were identified by the FT-IR studies and the particle size were determined. The drug polymer compatibility studies were determined by using FT-IR study and found out no interaction between the drug and polymers. Colon targeted drug delivery system of mesalamine tablets were prepared by using different formula by wet and dry granulation method, the successful batches were determined. All the prepared granules were evaluated for the flow properties by studied the angle of repose, compressibility index and hausner's ratio. The prepared granules were studied for the particle size determination by using Malvern particle size analyzer. The prepared tablets were evaluated the hardness, friability, weight variation and disintegration studies. The prepared tablets were coated and the optimized coating formula were analyzed. The enteric coated tablets were analyzed for the disintegration, dissolution and drug content

Sanket D Gandhi et al., (2010) the objective of the present study is to develop colon targeted drug delivery system for ivermectin using guar gum as a carrier in the treatment of helminthiasis. Matrix tablets containing various proportions of guar gum were prepared by wet granulation technique using starch paste as a binder. The idea was that the enteric coating would prevent drug release and absorption in the upper gastro intestinal tract. All the formulations were

evaluated for hardness, drug content uniformity and were subjected to in vitro drug release studies. The amount of ivermectin released from the matrix tablets at different time intervals was estimated by a UV Spectroscopy method. Colon targeted matrix tablet of ivermectin containing Guar gum released no ivermectin in the physiological environment of stomach (0.1N HCL) and small intestine (phosphate buffer 7.4pH). When the dissolution study was continued in simulated colonic fluids (Phosphate buffer 6.8 pH) the matrix tablets released.

AIM AND OBJECTIVE OF THE STUDY

AIM:

To prepare and evaluate chitosan nanoparticles loaded capecitabine drug formulation used for colorectal cancer.

OBJECTIVES:

- To prepare capecitabine nanoparticles by ionic gelation method.
- To study the particle size and morphology of prepared capecitabine nanoparticles.
- To formulate prepared nanoparticles into tablets.
- To evaluate the tablets.
- To study the *in-vitro* release of prepared tablets.

PLAN OF WORK

Phase: 1

- Drug profile
- Polymer profile
- Excipient profile
- Preformulation studies

Phase: 2

- Preparation of capecitabine nanoparticulate formulation by ionic gelation method.

Phase: 3

Characterization of nanoparticulate formulation

- Particle size determination
- Zeta potential determination
- Phase contrast microscopy of nanoparticles
- Scanning electron microscopy
- Drug content and entrapment of formulation

Phase: 4

- Evaluation of flow properties of freeze dried products.

Phase: 5

- Preparation of enteric coated tablets of capecitabine nanoformulation using CODESTM technology.

Phase :6

- Weight variation
- Hardness test
- Thickness test

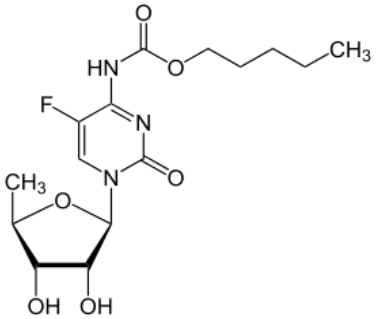
- Friability test
- *Invitro* release study
- Drug release kinetics

Phase :7

- Cell line studies.

DRUG PROFILE

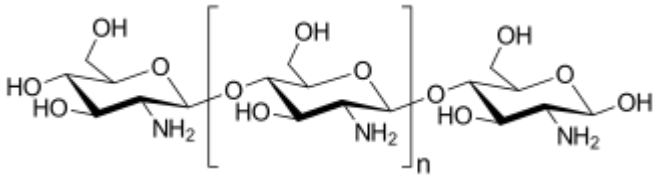
Drug name	Capecitabine
Synonym	5'-Deoxy-5-fluoro-N [(pentyloxy)carbonyl]-cytidine
Common name	Xeloda
Classification	Antimetabolite
IUPAC name	pentyl N-{1-[(2R, 3R, 4S, 5R)-3, 4-dihydroxy-5-methyloxolan-2- yl] -5- fluoro-2-oxo-1, 2-dihydropyrimidin-4-yl} carbamate.
CAS number	154361-50-9
Weight	Average: 359.3501 Mono isotopic: 359.149263656
Chemical formula	$C_{15}H_{22}FN_3O_6$

Structure	
Description	Capecitabine is a pro drug that is enzymatically converted to fluorouracil (anti-metabolite) in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. This compound belongs to the class of organic compounds known as glycosylamines. These are compounds consisting of an amine with a beta-N-glycosidic bond to a carbohydrate, thus forming a cyclic hemiaminal ether bond.
Indications	For the treatment of patients with metastatic breast cancer resistant to both paclitaxel and an anthracycline-containing chemotherapy regimen. It can also be used in combination with docetaxel for the treatment of metastatic breast cancer in patients, who have failed to respond to, or recurred or relapsed during or following anthracycline-containing chemotherapy. Capecitabine is used alone as an adjuvant therapy following the complete resection of primary tumor in patients with stage III colon cancer when mono therapy with fluropyrimidine is preferred. The use or capecitabine in combination regimens for advanced gastric cancer is currently being investigated.
Route of administration	Oral
Mechanism of action	Capecitabine is a prodrug that is selectively tumor-activated to its cytotoxic moiety, fluorouracil, by thymidine phosphorylase, an enzyme found in higher concentrations in many tumors compared to normal tissues or plasma. Fluorouracil is further metabolized to two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP), within normal and tumor cells. These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor, N5-10-methylene tetra hydrofolate, bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2'-deaxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is

	essential for the synthesis of DNA, therefore a deficiency of this compound can inhibit cell division. Secondly, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA.
Absorption	Readily absorbed through the GI tract (~70%). After oral administration, capecitabine is rapidly taken up from the gut and converted into its main metabolites 5-deoxy-5-fluoro cytidine (5- DFCR) and 5-deoxy-5-fluorouridine (5 - DFUR). Systemic levels of 5-FU are low. Concomitant food intake significantly reduces the systemic exposure to capecitabine. It is recommended to take the drug after a meal because this has also been done in the clinical trials. The time to reach the maximal plasma concentration after food ingestion is around 2 hours. Oral pharmacokinetics are linear. The absolute bioavailability is estimated to be 40%–45%
Protein binding	Binding is mainly to albumin and is 54% for capecitabine and 10%, 62%, and 10% for its metabolites 5 - DFCR, 5 - DFUR, and 5-FU, respectively. No relevant interactions at this level are to be expected.
Metabolism	After oral uptake, capecitabine is first metabolized to 5 - DFCR, which takes place mainly in the liver by carboxyl-esterase. The metabolite is converted to 5-DFUR by cytidine deaminase in liver and tumor tissue and converted to 5-FU intracellularly by thymidine phosphorylase, an enzyme that is often expressed in tumor tissue. Catalytic inactivation of 5-FU proceeds by dihydro pyrimidine dehydrogenase (DPD), which is poly morphically express.
Elimination	Capecitabine and its metabolites are predominantly excreted in urine; 95.5% of administered capecitabine dose is recovered in urine. Fecal excretion is minimal (2.6%). About 3% of the administered dose is excreted in urine as unchanged drug.
Pharmacodynamics	Capecitabine is a fluropyrimidine carbamate with anti-neoplastic activity indicated for the treatment of metastatic breast cancer and colon cancer. It is an orally administered systemic pro drug that has little pharmacologic activity until it is converted to fluorouracil by enzymes that are expressed in higher concentrations in many tumors. Fluorouracil it then metabolized both normal and tumor cells to 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP).

Half- life	45-60 mins.
Pharmacogenetics	Dihydro pyrimidine dehydrogenase (DPD) deficiency may lead to significant toxicity.
Toxicities	Hand foot syndrome, Diarrhea, Mucositis, Nausea, Fatigue, Bone marrow toxicity (less common), Angina like chest pain (rare)
Uses	Colorectal cancer Gastric cancer Breast cancer Esophageal cancer

POLYMER PROFILE

Polymer	Chitosan
Non proprietary name	BP : chitosan Hcl PhEur: chitosanihydrochloridum
Synonym	2-amino-2-deoxy-(1,4)-b-d-glucopyranan; deacetylated chitin; deacetyl chitin; b-1,4–poly–D–glucosamine; poly-D-glucosamine; poly-(1,4)-b-D-glucopyranosamine.
Chemical name	Poly-b-(1,4)-2-Amino-2-deoxy-D-glucose.
Molecular formula	$C_{56}H_{103}N_9O_{39}$
Molecular weight	1526.464 g/mol
Structure	
Description	Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D–glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance like sodium hydroxide. Chitosan chitin are polysaccharide polymers containing more than 5000 glucosamine and acetyl glucosamine units, respectively and molecular weights are over one million daltons. Chitosan occurs as odourless, white creamy powder or flakes. Fibre formation is quite common during precipitation and chitosan may look like cotton. Chitosan is obtained by the alkaline deactivation of chitin.
Functional category	Coating agent, disintegrant, film forming agent, mucoadhesive, tablet binder, viscosity increasing agent.
Stability and storage	Chitosan powder is a stable material at room temperature,

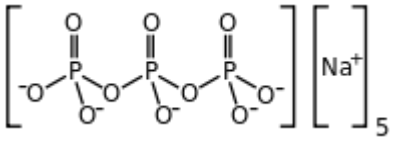
conditions	although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool dry place. The phEur 2005 specifies that chitosan should be stored at a temperature of 2-8˚c.
Incompatibilities	Chitosan is incompatible with strong oxidizing agents.
Safety	Chitosan is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. It is also used in cosmetics. Chitosan is generally regarded as a nontoxic and non-irritant material. It is biocompatible with both healthy and infected skin. Chitosan has been shown to be biodegradable.
Applications in pharmaceutical formulation	Chitosan is used in cosmetics and is under investigation for use in a number of formulations. The suitability and performance of chitosan as a component of pharmaceutical formulation for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications use as a component of mucoadhesive dosage forms, rapid release dosage forms improved peptide delivery, colonic drug delivery systems, and use for gene delivery. Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets and coatings for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spray drying, coacervation, direct compression and conventional granulation processes.
Solubility	Sparingly soluble in water, practically insoluble in ethanol (95%) other organic solvents, neutral or alkali solutions at pH above approximately 6.5 chitosan dissolve readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulphuric acids). The higher the ionic strength, the lower the

	solubility as a result of a salting out effect, which leads to the precipitation of chitosan in solution. Addition of an electrolyte reduces this effect and the molecule possesses a more random, coil-like conformation.
Acidity or alkalinity	pH=4.0-6.0 (1%W\V aqueous solution)
Density	1.35-1.40 g cm^{-3}
Glass transition temperature	203˚C
Moisture content	Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.
Particle size distribution	<30 mins.
Viscosity	A wide range of viscous types is commercially available owing to its high molecular weight and linear, unbranched structure, chitosan is an excellent viscosity enhancing agent in an acidic environment. The viscosity of chitosan solutions increases with increasing chitosan concentration, decreasing temperature, and increasing degree of deacetylation.
Uses	Chitosan has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and bio pesticide, helping plants to fight off fungal infections. In winemaking, it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.

EXCIPIENT PROFILE

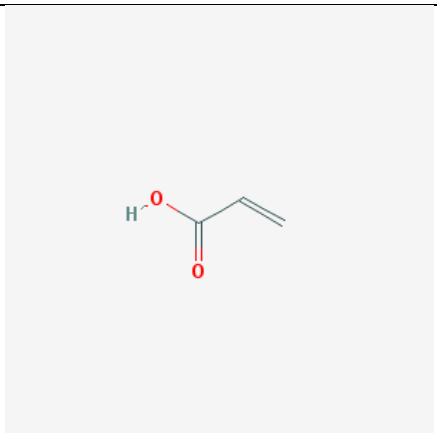
Excipient	Acetic acid
Nonproprietary name	BP : Glacial acetic acid
Synonyms	Acidumaceticumglaciale, E260, Ethanoic acid, ethylic acid, methane carboxylic acid, Vinegar acid.
Chemical name and CAS number	Ethanolic acid [64-19-7]
Molecular formula	$C_2H_2O_2$
Molecular weight	60.05
Structure	CH_3CH_2OH
Function	Acidifying agent
Description	Glacial acetic acid occurs as a crystalline mass or a clear, colorless volatile solution with a pungent odour.
Application	It is used in pharmaceutical products as a buffer system when combined with an acetate salt such as sodium acetate.
Boiling point	118˚C

Dissociation constant	pKa = 4.76
Melting point	17˚C
Refractive index	1.3718
Specific gravity	1.045
Solubility	Miscible with ethanol, ether, glycerin, water and other fixed and volatile oils.
Storage	It should be stored in an air tight container in a cool, dry place.
Incompatibilities	Acetic acid reacts with alkaline substances.
Related substances	Glacial acetic acid, dilute acetic acid, artificial vinegar.
Method of manufacture	Acetic acid is usually made by one of three routes: acetaldehyde oxidation, involving direct air or oxygen oxidation of liquid acetaldehyde in the presence of manganese acetate, cobalt acetate or copper acetate; liquid – phase oxidation of butane or naphtha; methanol carboxylation using a variety of techniques.
Handling precautions	Acetic acid, practically glacial acetic acid, can cause burns on contact with the skin, eyes, and mucous membranes. Protective clothing, eye protection are recommended.

Excipient	Tri polyphosphate
IUPAC name	Penta sodium triphosphate
Other names	Sodium tri polyphosphate, polygon
Structure	
CAS number	7758-29-4
Molecular formula	$Na_5P_3O_{10}$
Molecular weight	367.864 g/mol
Appearance	White powder.
Density	2.52 g/cm^3
Melting point	622˚C
Solubility	It dissolves in water.
Use	Chelating agent.

Excipient	Calcium chloride
Nonproprietary names	BP: Calcium chloride dihydrate Calcium chloride hexahydrate JP: Calcium chloride hydrate USP-NF: Calcium chloride
Synonyms	Calcium chloridum di hydricum, Calcii chloridum hexa hydricum
Chemical name and CAS number	Calcium chloride anhydrous[10043-52-4] Calcium chloride dihydrate[10035-04-8] Calcium chloride hexahydrate[7774-34-7]
Molecular formula and molecular weight	$Cacl_2$ - 110.98 (for anhydrous) $Cacl_2.2H_2O$ - 147.0 (for dihydrate) $Cacl_2.6H_2O$ - 219.1 (for hexahydrate)
Functions	Antimicrobial preservative, therapeutic agent, water-absorbing agent.
Description	Calcium chloride occurs as a white or colorless crystalline powder, granules, or crystalline mass, and is hygroscopic.
Application	It is an excipient relates to its dehydrating properties and, therefore, it has been used as an antimicrobial preservative, as a desicant, and as an astringent in eye lotions.
Boiling point	>1600˚C (anhydrous)
Density	0.835 g/cm^3 (dihydrate)
Melting point	772˚C (anhydrous) 176˚C (dihydrate) 30˚C (hexahydrate)
Solubility	Freely soluble in water and ethanol (95%), insoluble in diethyl ether.

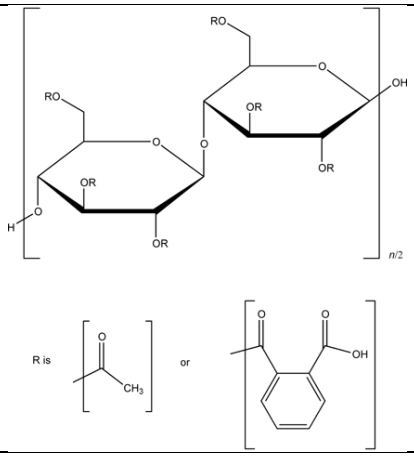
Stability	Calcium chloride is chemically stable; however, it should be protected from moisture.
Storage	Store in airtight containers in a cool, dry place.
Incompatibilities	It is incompatible with soluble carbonates, phosphates, sulfates and tartarates.
Method of manufacture	It is a byproduct from the Solvay process.
Handling precautions	Calcium chloride is irritating to eyes, the respiratory system, and skin. Gloves, eye protection, respirator and other protective clothing should be worn.

Polymer	Carbopol
Nonproprietary names	BP: Carbomers PhEur: Carbomers USP-NF: Carbomer
Synonyms	Acrypol, Acritamer, Acrylic acid polymer, Carbomera, Carbopol, Carboxy polymethylene, Polyacrylic acid, Carboxyvinyl polymer, Pemulen, Carbomer
Chemical Name	Carbomer [9003-01-4]
CAS Number	Alternative CAS registry numbers have been used for carbomer 934 [9007-16-3], 940 [9007-17-4], and 941 [9062-04-08]. The CAS registry number [9007-20-9] has also been used for carbomer
IUPAC Name	prop-2-enoic acid
Structure	
Molecular formula	$C_3H_4O_2$ CH_2=CHCOOH
Molecular weight	72.063 g/mol
Description	Carbomers are white-coloured, fluffy, acidic, hygroscopic powders with a characteristic slight odour. A granular carbomer is also available.
Functions	Bioadhesive material, controlled release agent, emulsifying agent, emulsion stabilizer, rheology modifier, stabilizing agent, suspending agent, tablet binder

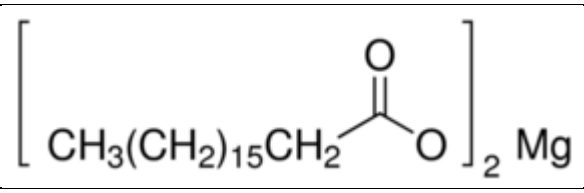
Applications	Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Formulations include creams, gels, lotions and ointments for use in ophthalmic, rectal, topical and vaginal preparations. It may used in oral preparations, in suspensions, capsules or tablets.
Density	Bulk:0.2g/cm^3 (powder), 0.4g/cm^3 (granular) Tapped:0.3g/cm^3(powder),0.4g/cm^3 (granular)
Dissociation constant	pKa=6.0±0.5
Glass transition temperature	100-105˚C
Melting point	Decomposition occurs within 30 minutes at 260˚C
Moisture content	Typical water content is up to 2%w/w. However, carbomers are hygroscopic and typical equilibrium moisture content at 25˚C and 50% relative humidity is 8-10%w/w. The moisture content of a carbomer does not affect its thickening efficiency, but an increase in the moisture content makes the carbomer more difficult to handle because it is less readily dispersed.
Particle size distribution	Primary particles average about 0.2 µm in diameter. The flocculated powder particles average 2-7 µm in diameter and cannot be broken down into the primary particles. A granular carbomer has a particle size in the range 150-425 µm.
Log P	0.35
Solubility	Swellable in water and glycerin and, after neutralization, in ethanol(95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally cross linked microgels.
Specific gravity	1.41
Viscosity	Carbomers disperse in water to form acidic colloidal dispersions that, when neutralized, produce highly viscous gels. Neutralized aqueous gels are more viscous at pH 6-11.The viscosity is considerably reduced at pH values less than 3 or greater than 12 or in the presence of strong electrolytes.

Stability	Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104˚C for up to 2 hours without affecting their thickening efficiency.
Storage	Carbomer powder should be stored in an airtight, corrosion resistant container and protected from moisture. The use of glass, plastic, or resin-lined containers is recommended for the storage of formulations containing carbomer.
Incompatibilities	Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes.
Method of manufacture	Carbomers are synthetic, high molecular weight, crosslinked polymers of acrylic acid. These acrylic acid polymers are cross linked with allyl sucrose or allyl penta erythritol..
Related substance	Polycarbophil
Handling precautions	Excessive dust generation should be minimized to avoid the risk of explosion. Carbomer dust is irritating to the eyes, mucous membranes and respiratory tract. In the event of eye contact with carbomer dust, saline should be used for irrigation purposes. Gloves, eye protection, and a dust respirator are recommended during handling.

Polymer	Cellulose acetate phthalate
Nonproprietary names	BP:Cellacefate JP:Cellacefate PhEur:Cellulose Acetate Phthalate USP-NF:Cellacefate
Molecular weight	80,000-1,40,000
Synonyms	Cellacefate; CAP; Celacefato; Cellacefato; Cellacefatum; Cellulose acetate hydrogen phthalate; cellulose acetate hydrogen 1,2- benzenedicarboxylate; Cellulose Acetate Monophthalate; Cellulose Acetylphthalate; Cellulose Acetophthalate; Cellulose acetate phthalate; HPMCP; Hypromellose Phthalate; Acetyl phthalyl cellulose; Cellacephate; Cellulose; Cellulose acetate monophthalate; Cellulose acetate phthalate; Cellulose aceto phthalate; Cellulose acetyl phthalate; Cellulosum
Method of Manufacture	Cellulose acetate phthalate is produced by reacting the partial acetate ester of cellulose with phthalic anhydride in the presence of a tertiary organic base such as pyridine, or a strong acid such as sulfuric acid.
Solubility	Soluble in most organic solvents, Insolube in hot water, alcohol
Stability	Stable under ordinary conditions.

Storage	Cellulose acetate phthalate is stable if stored in a well-closed container in a cool, dry place.
Appearance	white to off-white powder or flakes
Viscosity	A 15%w/w solution in acetone with a moisture content of 0.4%. This is a good coating solution with a honey-like consistency, but the viscosity is influenced by the purity of the solvent.
Melting point	192°C. Glass transition temperature is 160–170°C.
Chemical name	Cellulose, acetate, 1,2-benzenedicarboxylate
CAS Number	9004-38-0
Structure	
Description	Cellulose acetate phthalate occurs as a hygroscopic, white to off-white, free-flowing powder, granule, or flake. It is tasteless and odorless, or might have a slight odor of acetic acid.
Functions	Coating agent; microencapsulating agent; tablet and capsule binder

Density	Density (bulk) – 0.260 g/cm3 Density (tapped) -0.266 g/cm3
Incompatibilities	Cellulose acetate phthalate is incompatible with ferrous sulfate, ferric chloride, silver nitrate, sodium citrate, aluminum sulfate, calcium chloride, mercuric chloride, barium nitrate, basic lead acetate, and strong oxidizing agents such as strong alkalis and acids.
Applications	Cellulose acetate phthalate (CAP) is used as an enteric film coating material, or as a matrix binder for tablets and capsules. Cellulose acetate phthalate is commonly applied to solid-dosage forms either by coating from organic or aqueous solvent systems or by direct compression. The addition of plasticizers improves the water resistance of this coating material, and formulations using such plasticizers are more effective than when cellulose acetate phthalate is used alone. It is also used in combination with other coating agents such as ethyl cellulose, in drug controlled-release preparations.
Related Substances	Cellulose acetate, hypromellose phthalate, polyvinyl acetate phthalate.
Safety	Cellulose acetate phthalate is widely used in oral pharmaceutical products and is generally regarded as a nontoxic material, free of adverse effects. However, it may be irritant to the eyes, mucous membranes, and upper respiratory tract.
Handling precautions	Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended.

Excipient	Magnesium stearate
Synonyms	Dibasic magnesium stearate, Magnesium distearate, Magnesiistearas, Magnesium octadecanoate, Octadecanoic acid, Magnesium salt, Stearic acid, Synpro 90
Chemical name	Octadecanoic acid magnesium salt
CAS Number	[557-04-0]
Molecular formula	$[CH_3(CH_2)_{16}COO]_2Mg$
Molecular weight	591.24
Structure	
Functions	Tablet and capsule lubricant
Description	Magnesium stearate is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint odour of stearic acid and a characteristic taste.The powder is greasy to the touch and readily adheres to the skin.
Application	Magnesium stearate is widely used in cosmetics, foods, and pharmaceutical formulations. It is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0%w/w. It is also used in barrier creams.
Density	Bulk:0.159g/cm^3 Tapped:0.286g/cm^3 True:1.092g/cm^3
Flowability	Poorly flowing, Cohesive powder
Melting point	117-150˚C

Solubility	Practically insoluble in ethanol, ethanol (95%), ether and water, slightly soluble in warm benzene and warm ethanol (95%)
Specific surface area	1.6-14.8m^2/g
Stability	It is stable
Storage	It should be stored in a well-closed container in a cool, dry place.
Related Compounds	Calcium stearate, magnesium aluminium silicate, stearic acid, zincs stearate.
Incompatibilities	Incompatible with strong acids, alkalis, and iron salts. Avoid mixing with strong oxidizing materials. Magnesium stearate cannot be used in products containing aspirin, some vitamins, and most alkaloidal salts.
Method of manufacture	Magnesium stearate is prepared either by the interaction of aqueous solutions of magnesium chloride with sodium stearate or by the interaction of magnesium oxide, hydroxide, or carbonate with stearic acid at elevated temperatures.
Handling precautions	Eye protection and gloves are recommended. Excessive inhalation of magnesium stearate dust may cause upper respiratory tract discomfort, coughing, and choking. Magneisum stearate should be handled in a well-ventilated environment.

Excipient	Talc
Synonyms	Altalc, Hydrous magnesium calcium silicate, Hydrous magnesium silicate, Imperial, Magnesium hydrogen metasilicate, Magsil Star, Powdered talc, Purified French chalk, Purtalc, Soapstone, Steatite
Chemical name	Talc
CAS number	[14807-96-6]
Molecular formula	$Mg_6(Si_2O_5)_4(OH)_4$
Molecular weight	It may contain small, variable amounts of aluminium silicate and iron.
Functions	Anticaking agent, glidant, tablet and capsule diluent, tablet and capsule lubricant
Description	Talc is a very fine, white to greyish-white, odorless, impalpable, crystalline powder. It adheres readily to the skin and is soft to the touch and free from grittiness.
Application	Talc was once widely used in oral solid dosage formulations as a lubricant and diluent.
Acidity/Alkalinity	pH=7-10 for a 20%w/v aqueous dispersion.
Moisture content	Talc absorbs insignificant amounts of water at 25˚C and relative humidities up tp about 90%.

Particle size distribution	Varies with the source and grade of material. Two typical grades are ≥99% through a 74µm (#200 mesh) or ≥99% through a 44µm (#325mesh).
Refractive index	1.54-1.59
Solubility	Practically insoluble in dilute acids and alkalis, organic solvents and water.
Specific gravity	2.7-2.8
Specific surface area	2.41-2.42m^2/g
Stability	Talc is a stable material and may be sterilized by heating at 160˚C for not less than 1 hour. It may also be sterilized by exposure to ethylene oxide or gamma irradiation
Storage	Talc should be stored in a well-closed container in a cool, dry place.
Incmpatibilities	Incompatible with quaternary ammonium compounds.
Related substances	Bectonite, Magnesium aluminum silicate, Magnesium silicate, Magnesium trisilicate.
Method of manufacture	Talc is a naturally occurring hydro poly silicate mineral. It is pulverized before being subjected to flotation processes to remove various impurities such as asbestos; carbon; dolomite; iron oxide; and various other magnesium and carbonate minerals. Following this process, talc is finely powdered, treated with dilute hydrochloric acid, washed with water, and then dried.
Handling precautions	Talc is irritant if inhaled and prolonged excessive exposure may cause pneumoconiosis. Eye protection, gloves and a respirator are recommended.

MATERIALS AND EQUIPMENTS

Table 3: LIST OF CHEMICALS USED

S.NO	INGREDIENTS	VENDER
1.	Capecitabine	Gift sample
2.	Chitosan	Sigma Aldrich Chemie USA
3.	Tri poly phosphate	Loba Chemie Ltd., Mumbai
4.	Calcium chloride	Loba Chemie Ltd., Mumbai
5.	Acetic acid	Thermo Fisher Scientific India Pvt. LTD
6.	Carbopol	Himedia Laboratories
7.	Cellulose acetate phthalate	Sigma Aldrich Chemie USA
8.	Ethanol	JEBSEN & JESSEN GmbH & CO Germany
9.	Acetone	S D Fine – Chem Limited
10.	Sodium hydroxide	Merch
11.	Potassium dihydrogen phosphate	Himedia Laboratories

Table 4: LIST OF EQUIPMENTS USED

1.	Digital Weighing Balance	Shimadzu, AZ 220
2.	Magnetic Stirrer	Remi Equioments Ltd, 1MLH
3.	Bath Sonicator	PCI, ultrasonics
4.	Centrifuge	Remi R-8c Laboratory
5.	pH meter	Systronics, pH system 361
6.	UV Spectrophotometer	UV-1650 Pc Shimadzu
7.	FT-IR Spectrophotometer	Shimadzu 8400 S
8.	Freeze drier	Delvac pumps, MINI LYODEL 225 V
9.	Zeta sizer	Nano ZS90, Malvern
10.	Hardness	Dolphin
11.	Thickness	Dolphin
12.	Friability	INWEKA
13.	Dissolution test apparatus	LABINDIA DS 8000
14.	Disintegration tester	LABINBIA
15.	Scanning Electron Microscope	ZEISS SUPRA 40
16.	Phase Contrast Microscope	Nikkon Eclipse TS – 100

PREFORMULATION STUDIES

MELTING POINT DETERMINATION:

Digital melting point apparatus is used to determine the melting point of capecitabine by capillary method. One end of the capillary tube was sealed with gentle heat using Bunsen burner and then the small quantity of pure drug capecitabine was filled into the sealed capillary tube. Then this capillary tube with the pure drug was placed in a melting point viewer. The temperature at which drug gets melted is taken as melting point of drug.

DETERMINATION OF λ MAX:

PREPARATION OF STOCK A:

The capecitabine stock-A solution was prepared by dissolving 100 mg of drug in 100 ml of water to obtain a drug concentration of 1000 mcg/ml.

PREPARATION OF STOCK B:

From the above stock-A, 1 ml were taken and diluted to 100 ml to prepare stock B of concentration 10 mcg/ml.

DETERMINATION OF ANALYTICAL WAVELENGTH:

To form concentration of 1 mcg/ml, 1ml of stock B was diluted to 10 ml, then the solution was scanned using double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 200 nm to 400 nm.

STANDARD CURVE OF CAPECITABINE:

100 mg of drug was weighed and transferred to a 100 ml standard flask, diluted to 100 ml with phosphate buffer to prepare concentration of 1000 mcg/ml and sonicated for few minutes which were the standard stock solution. 1ml of this stock solution was taken and diluted with 100ml buffer to obtain concentration at 10 mcg/ml. And this prepared solution was serially diluted to get the different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10mcg/ml) to determine the linearity range. The standard samples were analyzed at 240 nm using UV Spectrophotometer.

SOLUBILITY STUDY:

Solubility of the drug is predicted by dissolving 1 mg of the drug in proportions of 1 ml, 10 ml, 30 ml and 100 ml of the proposed solvents. So according to the dilution or the dissolving property the solubility was predicted by measuring 240 nm by UV-Visible spectroscopy. A test for the solubility becomes a test for purity only where a special quantitative test is given in the individual monograph and is an official requirement. (IP [2.4.26 Solubility])

SOLUBILITY PARAMETERS:

Table 5: SOLUBILITY PARAMETERS

DESCRIPTIVE TERMS	**PART OF SOLVENT REQUIRED FOR PART OF SOLUTE**
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10000
Practically insoluble	10000 or more

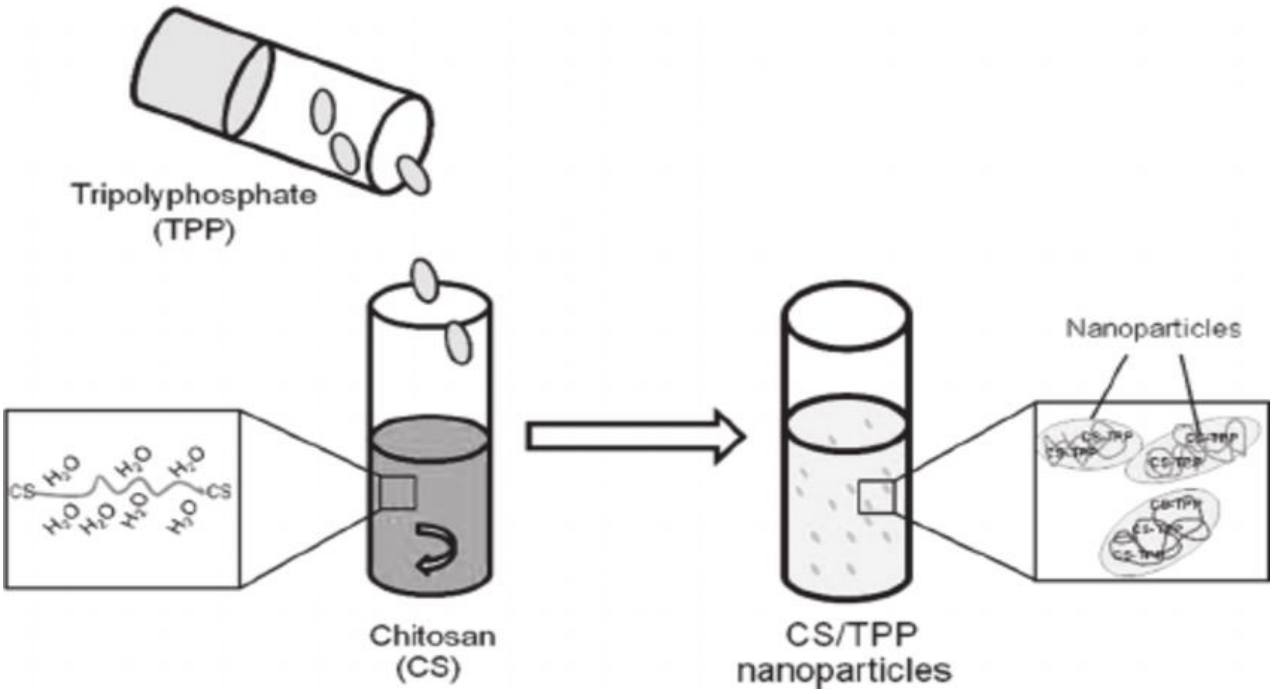
FT – IR DRUG POLYMER COMPATIBILITY STUDY:

Infrared spectra of drug and its inclusion complexes were recorded by KBr pellet method using Fourier Transform Infrared spectrophotometer. A base line correction was made using dried potassium bromide and then spectra of dried mixtures of drug and inclusion complexes with potassium bromide were recorded. The samples were prepared by KBr pellet press method; the scanning wavelength range was 400-4000 cm-1.

METHODOLOGY

FORMULATION OF CAPECITABINE NANOPARTICLES:

Figure 7: IONIC GELATION METHOD



a) Preparation of chitosan solution:

Chitosan was weighed and transferred into a glass beaker. To this chitosan, 0.1ml of acetic acid and 50ml of water was added and kept for overnight stirring to obtain product A.

b) Preparation of tri polyphosphate solution:

Tri polyphosphate was added to the distilled water. To this solution, drug solution was added and kept for 30 minutes stirring to obtain product B.

c) Formulation of capecitabine nanoparticles:

The product A (chitosan solution) was taken in a beaker and kept for continuous stirring. Then the drug solution was taken in an insulin syringe and added drop by drop to the chitosan solution. The final solution was mixed with $CaCl_2$. Finally the capecitabine nanoparticle was prepared.

CHARACTERIZATION OF CAPECITABINE NANOSUSPENSION:

a) Determination of particle size by photon correlation spectroscopy:

The average mean diameters and size distribution of nanosuspension was found out by photon correlation spectroscopy using Zetasizer (nano ZS90, Malvern Instruments) at 25˚C.The samples were kept in polystyrene cuvette and the readings were find out at a fixed angle

b) Determination of zeta potential:

The electrophoretic mobility (zeta potential) measurements of nanosuspension were made using Zetasizer (Nano ZS90, Malvern Instruments). The samples were placed in a polystyrene cuvette (at 25˚C) and Zeta dip cell was used to measure the potential.

c) Lyophilization technique:

Freeze drying was performed to convert the aqueous solution of drug loaded nanoparticles into powder. The selected formulations were kept in well closed containers and allowed to freeze under -80˚C for 4 hours. Then the formulation was freeze dried using LYODOL freeze dryer at -40˚C and -1.12 mbar pressure for a period of 72 hours.

CHARACTERIZATION OF CAPECITABINE NANOPARTICLE:

SCANNING ELECTRON MICROSCOPY: (SEM)

The shape and surface characteristics was observed by scanning electron microscopy (SEM). The morphology of the lyophilized capecitabine loaded nanoparticles was characterized by using field emission scanning electron microscopy (Zeiss Supra 40 apparatus Germini column, Germany) operated at 5 KV accelerating voltage. Scanning electron microscopy is a type of electron microscopy that images the surface of solid specimen by using focused beam of high

energy electrons. The scanning process and image formation in SEM depends on signal produced by elastic and inelastic interactions between high energy electron beam and specimen surface. The particle size analysis of lyophilized nanoparticles was carried out to confirm the Nano size of the formulation. The samples were lightly sprinkled on a double sided adhesive tape stuck to an aluminium stub and the stubs were placed in scanning electron microscope chamber.

PHASE CONTRAST MICROSCOPY:

The samples was taken on glass slide and observed under Nikon Leica inverted phase contrast microscope. Images were taken on computer monitor using Leica software and shape of the particles was observed.

DRUG CONTENT IN THE FORMULATION:

22mg product was dissolved in 100ml of distilled water. From this 1ml was taken and made up to 10ml and absorbance was taken at 240nm using UV spectrophotometer. From this the required amount of nano formulation to be made into tablet and it is being calculated.

ENTRAPMENT EFFICIENCY:

2ml of the formulation was taken and ultra-centrifuged at 13,000rpm at 4˚C for 30minutes using eppendorf centrifuge. The supernatant was recovered using micro pipette.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100$$

FORMULATION OF CAPECITABINE LOADED CHITOSAN NANOPARTICLES:

Table 6: FORMULATION OF CAPECITABINE NANOPARTICLES

Formulation	Chitosan (mg)	Acetic acid (ml)	Water (ml)	Tri polyphosphate (mg)	Drug (mg)	Water (ml)	Calcium chloride (mg)
1	200	1	100	3.5	150	10	-
2	200	1	100	3.5	150	10	50
3	200	1	100	7.2	150	10	-
4	200	1	100	7.2	150	10	50
5	200	1	100	7.2	150	50	-
6	200	1	100	7.2	150	50	50
7	100	0.5	50	7.2	50	50	-
8	100	0.5	50	7.2	50	50	50
9	100	0.5	50	14.5	50	50	-
10	100	0.5	50	14.5	50	50	50

PREPARATION OF TABLETS:

Capecitabine tablets were prepared by direct compression method in which active ingredients and excipients are present. To the formulation product magnesium stearate and talc were added and the tablets were compressed. The formula for preparation of tablets was listed below.

Table 7: PREPARATION OF TABLETS

Ingredients	Amount per tablet(mg)
Formulation	645
Magnesium stearate (2%)	12.9
Talc (1%)	6.45
Total	664.35

EVALUATION OF TABLETS:

ANGLE OF REPOSE:

The method used to find the angle of repose is to pour the powder a conical on a level, flat surface and measure the included angle with the horizontal.

$$\theta = \text{Tan}^{-1} (h/r)$$

Where, θ = Angle of repose, h = Height of the powder cone, r = Radius of the powder cone.

BULK DENSITY AND TAPPED DENSITY OF GRANULES:

The bulk density and tapped bulk density were determined and calculated by the following formulas.

BD = weight of the powder / initial volume

TD = weight of the powder / final volume

COMPRESSIBILITY OF GRANULES:

The compressibility index was determined by Carr‟s compressibility index and Hausner‟s ratio.

Carr's index = TD – BD X 100 / BD

Hausner's ratio = TD / BD

WEIGHT VARIATION TEST:

20 tablets of formulation were weighed using electronic balance and the test was performed according to the USP official limits. Percentage deviation of tablet is calculated in the table below.

$$\%\text{Maximum positive deviation} = [W_H - \frac{A}{A}] \times 100$$

$$\%\text{Minimum negative deviation} = [A - \frac{WL}{A}] \times 100$$

Where,

W_H - Highest weight of tablet in mg

W_L - Lowest weight of tablet in mg

A - Average weight of tablet in mg

IP/BP AND USP LIMITS OF WEIGHT VARIATION TEST:

Table 8: LIMITS OF WEIGHT VARATION

IP/BP	USP	LIMIT
80mg or less	130mg or less	±10%
More than 80mg and less than 250mg	130mg to 324mg	±7.5%
250mg or more	More than 324mg	±5%

TABLET THICKNESS:

Thickness of tablet was important for uniformity of tablet size. Tablets were selected and thickness was measured by using vernier – caliper scale, which permits accurate measurement.

HARDNESS OF TABLETS:

Hardness of tablet is the indication of its strength against resistance of tablet to capping, abrasion or breakage under conditions of storage, temperature and handling before usage. Hardness of tablets was determined using Pfizer hardness tester and is measured in kg/cm^2.

FRIABILITY TEST:

The friability of the tablets was determined by Roche friabilator. Accurately weigh the tablets and place them in the friabilator. These tablets were subjected to friability at 25rpm for 4 mins (100 rotations). Initial and final weights of the tablets were noted and calculated as per following formula,

$$\% \text{ Friability} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

DISINTEGRATION TEST:

Disintegration test for the prepared tablets were performed using Basket-rack assembly. One tablet was placed in each of six tubes of the basket and test was performed using different buffers as the immersion fluid maintained at 37± 2˚C. Time for complete disintegration of all six tablets was noted.

DISSOLUTION TEST:

Dissolution studies were carried out by using USP-II dissolution test apparatus using paddle method. For dissolution testing tablets, immersed at a time, and evaluated in pH 1.2, 7.4, and 6.8 dissolution media. To match the changes in pH along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were used sequentially. These three media represents the stomach, proximal part of the small intestine and terminal ileum respectively. When performing studies, the pH 1.2 medium was first used for 2 h, and then replaced with the fresh pH 7.4 phosphate buffers. After 3-4 hour the medium was again replaced with fresh pH 6.8 dissolution medium.

CELL LINE STUDIES:

Cell culture:

The colorectal cancer cell lines (HT 29) were purchased from the National Center for Cell Sciences (NCCS), Pune, India. The cells were maintained in their respective modified eagles medium (MEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na2CO3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100 µg) were adjusted to 1mL/L. The cells were maintained at 37◦C with 5% CO_2 in a humidified CO_2 incubator.

Evaluation of cytotoxicity:

The inhibitory concentration (IC50) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were grown (1×104cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced

with fresh medium containing serially diluted compounds (Samples), and the cells were further incubated for up to 48 h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37◦C for 4 h. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multi well plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

$$\text{Formula: \% of viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

DRUG RELEASE STUDIES:

Mathematical model plays important role in the prediction of mechanism of drug release and also provides more general guidelines for development of other system. To describe the drug release rate from different drug delivery system a large numbers of models were developed. Some of important models are,

- Zero order kinetic model
- First order kinetic model
- Higuchi model
- Korsmeyer-peppas model

The model that best fits the release data is selected based on the correlation coefficient value in various models. The model that gives high R^2 value is considered as the best fit of the release data.

Zero order release:

It describes the systems where the drug release rate is independent of its concentration of the dissolved substance.

$Q_t = Q_0 + K_0 t$

Where,

Q_0 = Initial amount of drug

Q_t = Cumulative amount of drug release at time "t"

K_o = Zero order release constant

t = Time in hours

First order release:

The drug release rate depends on its concentration.

$$\text{Log } Q_t = \text{Log } Q_o + K_t/2.303$$

Where,

Q_o = Initial amount of drug

Q_t = Cumulative amount of drug release at time "t"

K = First order release constant

t = Time in hours

Higuchi release:

The Higuchi equation suggests that the drug release by diffusion.

Where,

Q = Cumulative amount of drug release at time "t"

K_H = Higuchi constant

t = Time in hours

Korsmeyer – peppas equation:

Korsmeyar – peppas equation is

$$F = (M_t/M) = K_m t^n$$

Where,

F = Fraction of drug released at time‘t’

M_t = Amount of drug released at time‘t’

M = Total amount of drug in dosage form

K_m = Kinetic constant

n = Diffusion or release exponent

t = Time in hours

RESULT AND DISCUSSION

PREFORMULATION STUDIES:

Melting point:

Melting point was found to be 121˚ C which confirms the drug capecitabine.

Solubility:

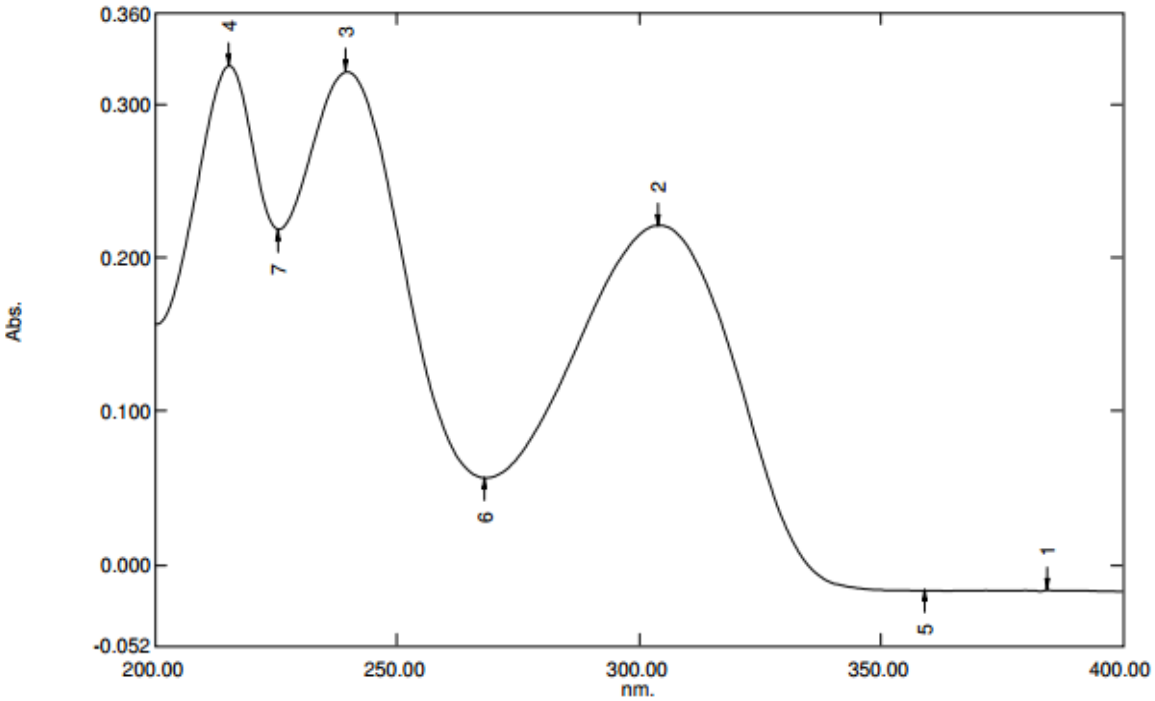
Solubility of capecitabine in different solvent was carried out and found that

- Soluble in water (26 mg/ml)
- Slightly soluble in ethanol (207 mg/ml)

Determination of lamda max of Capecitabine:

The prepared stock solution of Capecitabine was scanned in UV – Visible spectrophotometer from 200-400 nm.

Figure 8: DETERMINATION OF LAMDA MAX BY UV SPECTROSCOPY



No.	P/V	Wavelength	Abs.	Description
1		384.60	-0.016	
2		303.80	0.221	
3		239.40	0.322	
4		215.20	0.326	
5		359.00	-0.017	
6		268.20	0.057	
7		225.40	0.219	

The maximum wavelength occurs at 239.40 nm and hence 240 nm was selected as maximum absorbance for further studies.

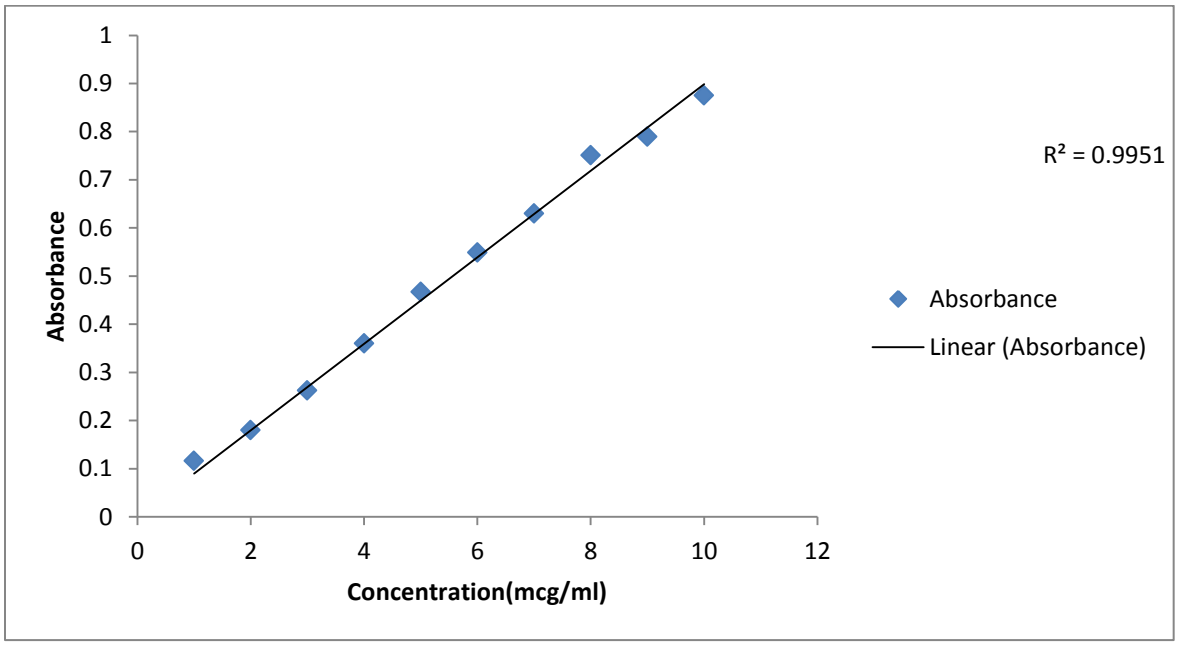
Calibration curve of Capecitabine by UV Spectrophotometer:

Standard curve of capecitabine was plotted by using UV spectrophotometer and the absorbance was noted as 240 nm. A series of concentrations of solutions ranging from 1-10 mcg/ml were prepared and the corresponding absorbance was noted. The absorbance values corresponding to the concentration were shown in table and the regression value was found to be 0.995 from the graph.

Table 9: STANDARD CURVE DATA OF CAPECITABINE

Concentration (mcg/ml)	Absorbance (240 nm)
1	0.116
2	0.180
3	0.262
4	0.360
5	0.467
6	0.549
7	0.630
8	0.751
9	0.789
10	0.875

Figure 9: STANDARD CURVE OF CAPECITABINE



FT – IR SPECTROSCOPY:

Figure 10: FT-IR OF CAPECITABINE

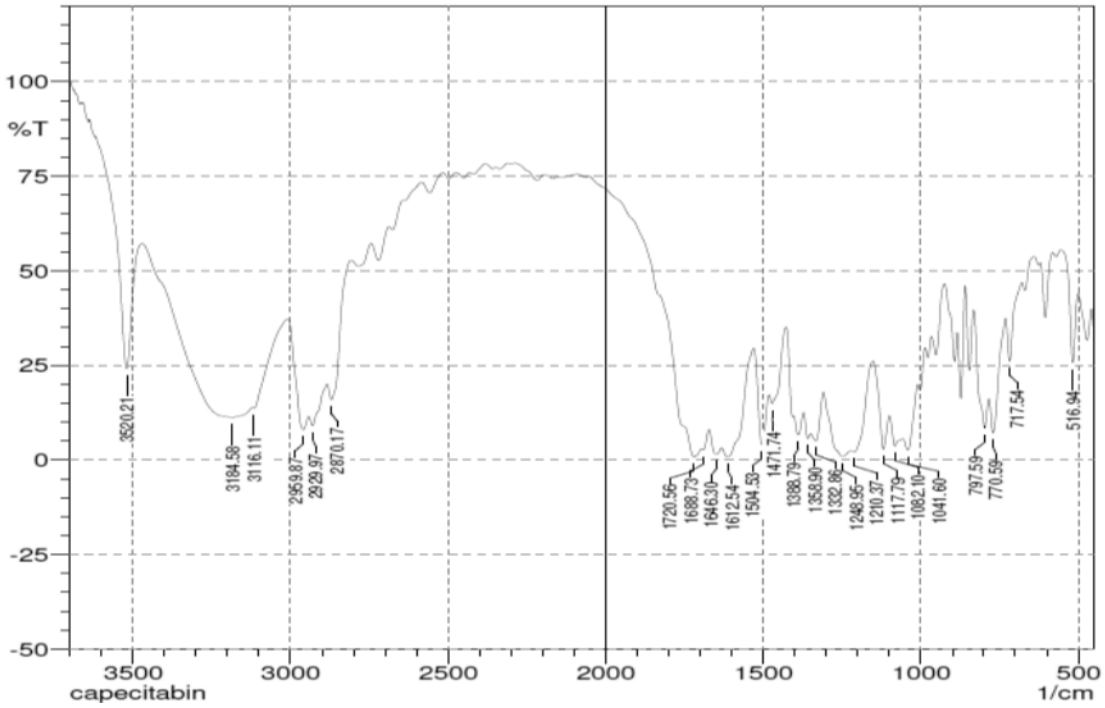


Table 10: FT-IR DATA OF CAPECITABINE

S. No	Wave Number cm^{-1}	Assignment
1	3184	O-H stretching
2	1552	C=O stretching
3	1720	C-F stretching

Figure 11: FT-IR OF CHITOSAN

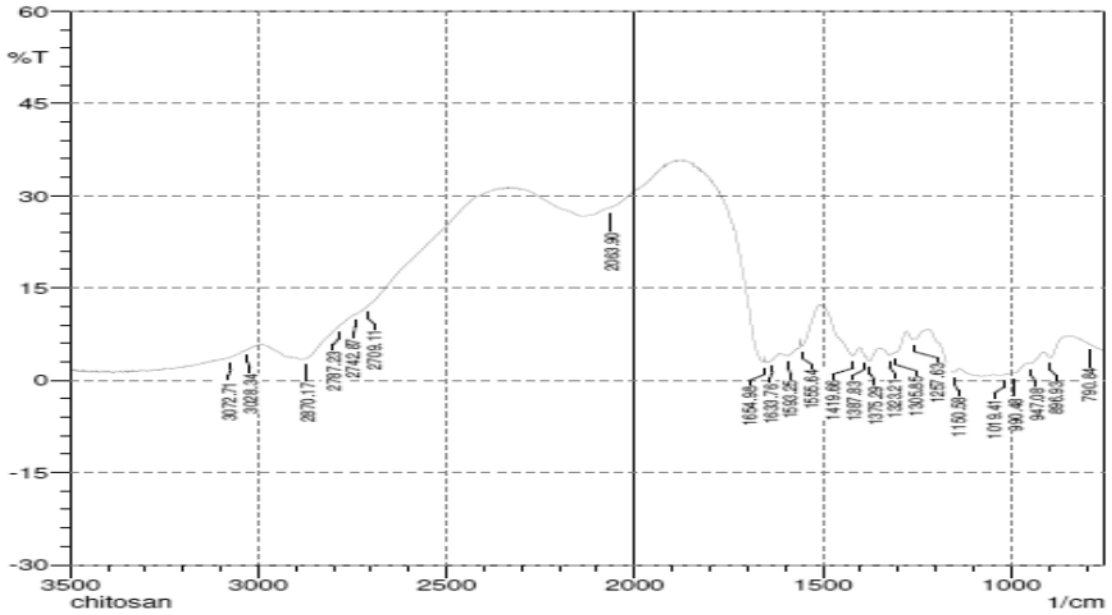


Table 11: FT-IR DATA OF CHITOSAN

S. No	Wave Number cm^{-1}	Assignment
1	1321	Carbonyl stretching of secondary amide
2	1152	N-H stretching of amine
3	1079	Ether bonds
4	1030,895	Secondary hydroxyl groups, characteristic peak of CH-OH and cyclic alcohols, C-0 stretching.

Figure 12: FT-IR OF CALCIUM CHLORIDE

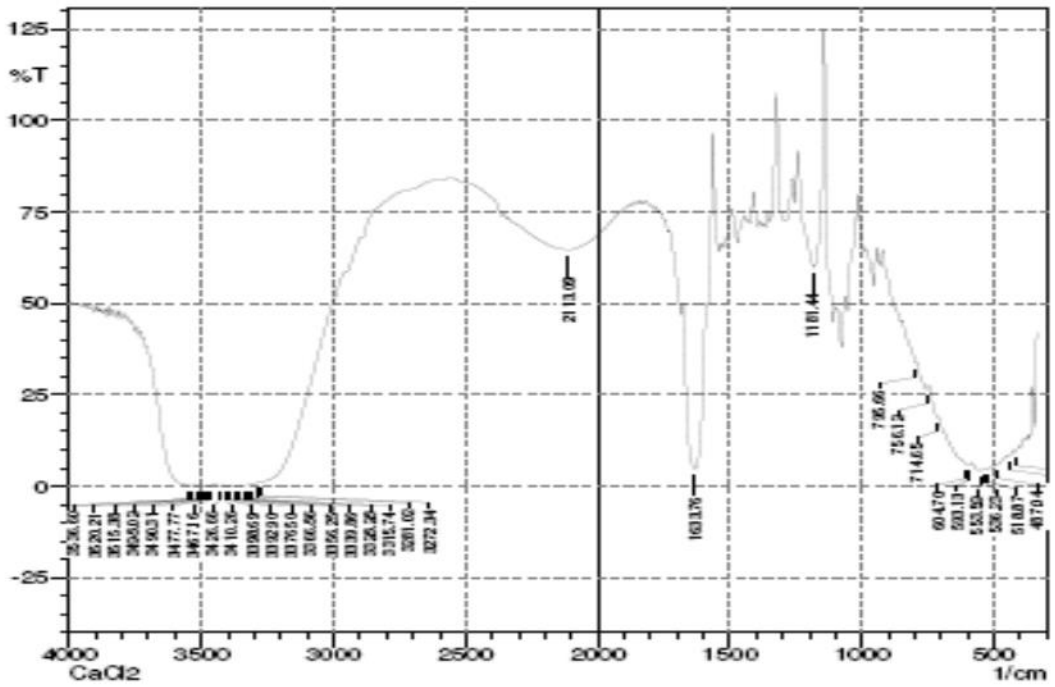


Table 12: FT-IR DATA OF CALCIUM CHLORIDE

S. No	Wave Number cm^{-1}	Assignment
1	3437	C-O-H stretching
2	3429	Asymmetric stretching peak of calcium salt group
3	602.9	Asymmetric stretching

Figure 13: FT-IR OF TRI POLYPHOSPHATE

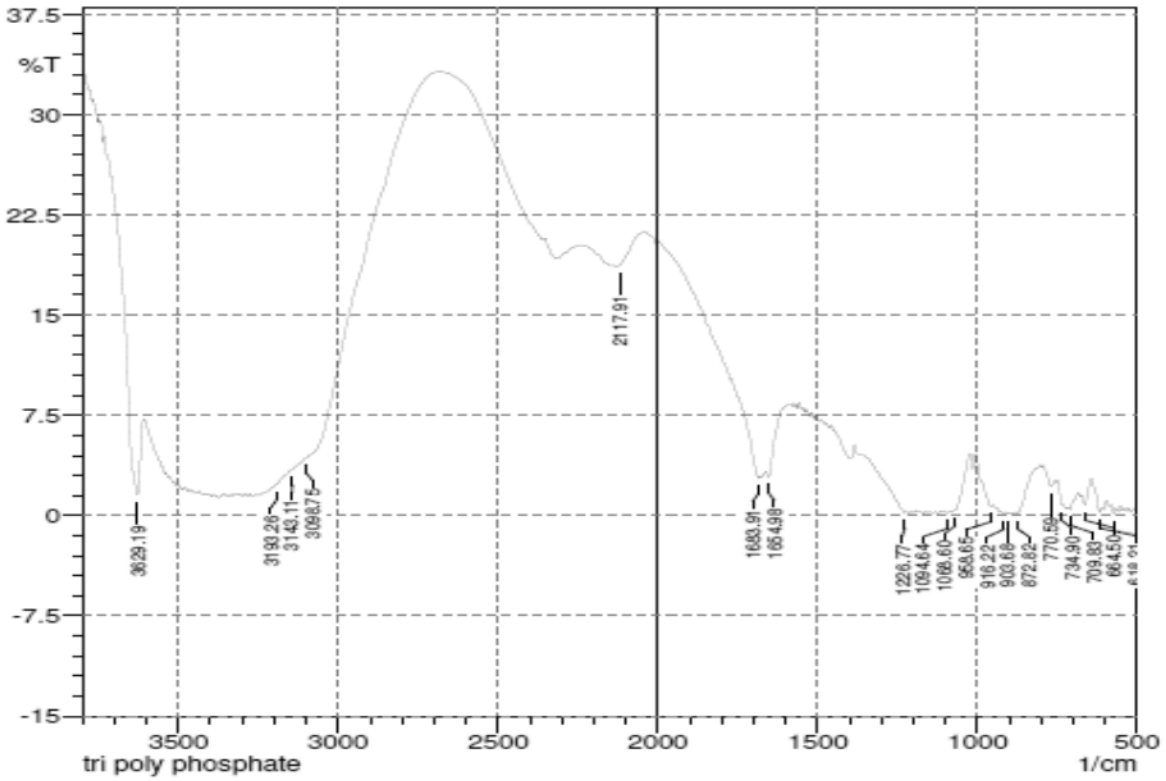


Table 13: FT-IR DATA OF TRIPOLYPHOSPHATE

S. No	Wave Number cm^{-1}	Assignment
1	1226	Secondary amide group stretching
2	1094	Symmetric stretching peak of C=C group

Figure 14: FT-IR OF MAGNESIUM STEARATE

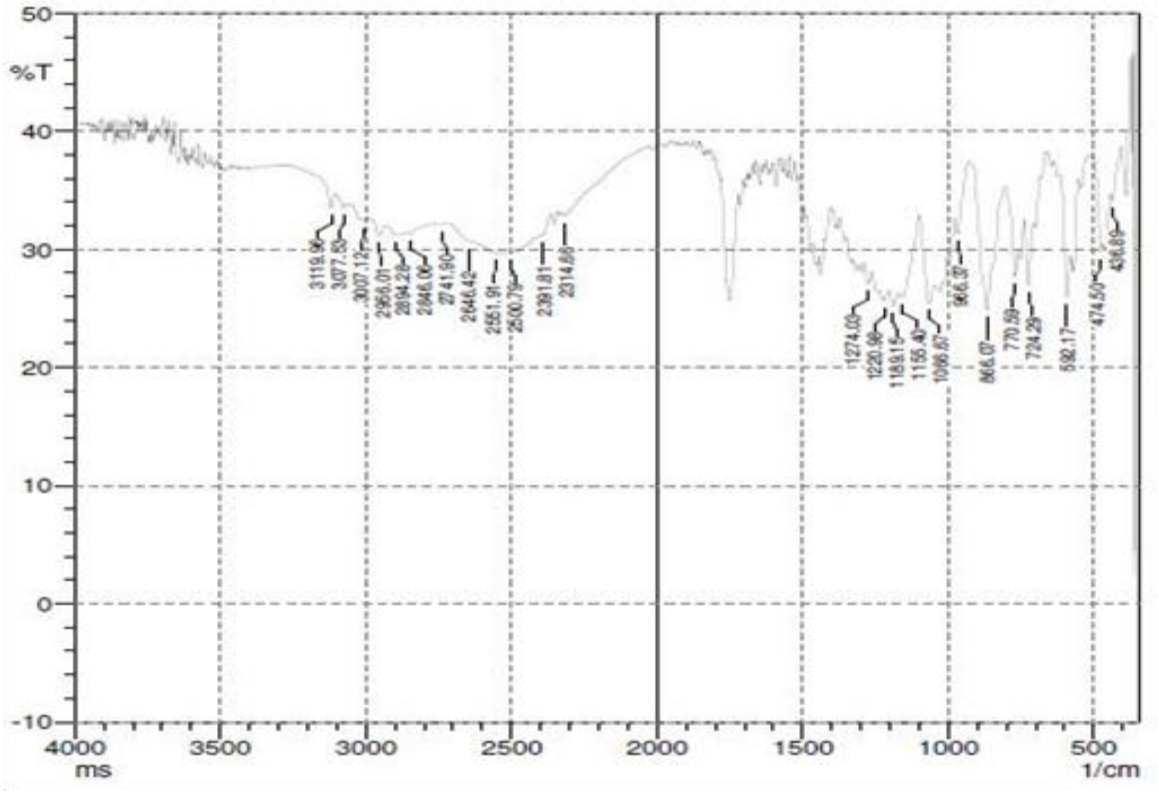


Table 14: FT-IR DATA OF MAGNESIUM STEARATE

S. No	Wave Number cm^{-1}	Assignment
1	2956	Symmetric C-H stretching
2	1550	C=O stretching
3	966	C-CH_3 bending

Figure 15: FT-IR OF TALC

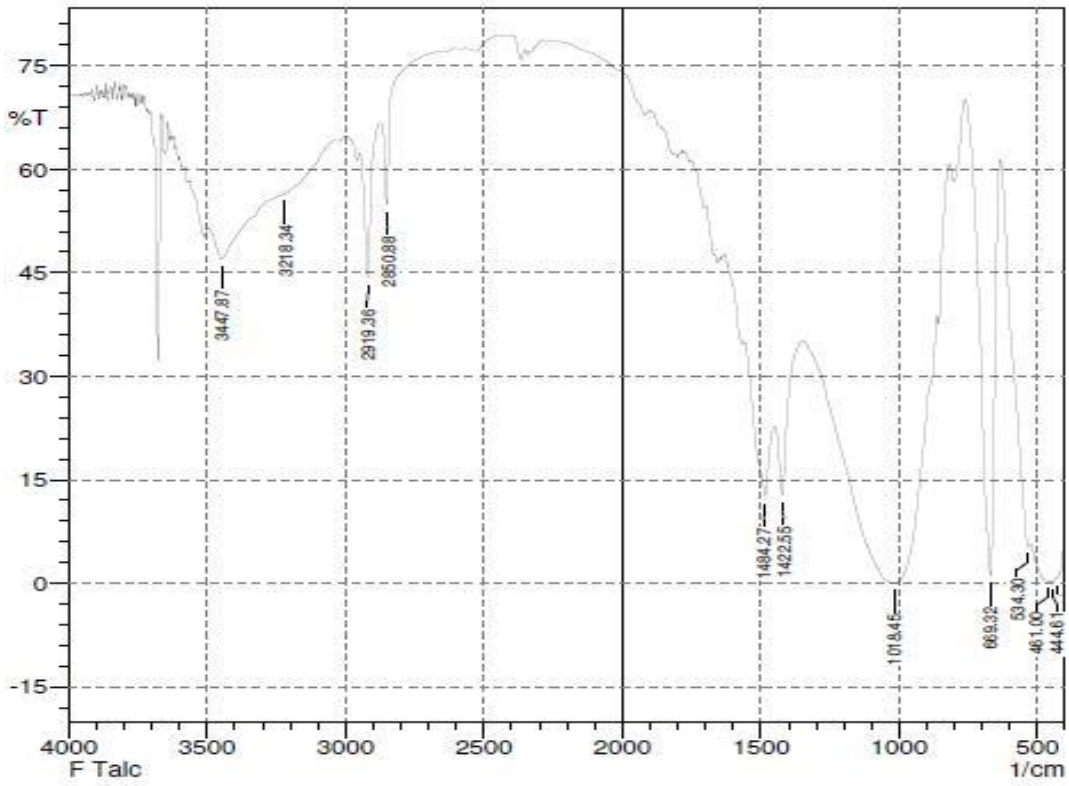


Table 15: FT-IR DATA OF TALC

S. No	Wave Number cm¯1	Assignment
1	2919	C-H stretching
2	2447	N-H stretching
3	1028	C-N bending

Figure 16: FT-IR OF PHYSICAL MIXTURE OF CAPECITABINE AND EXCIPIENTS

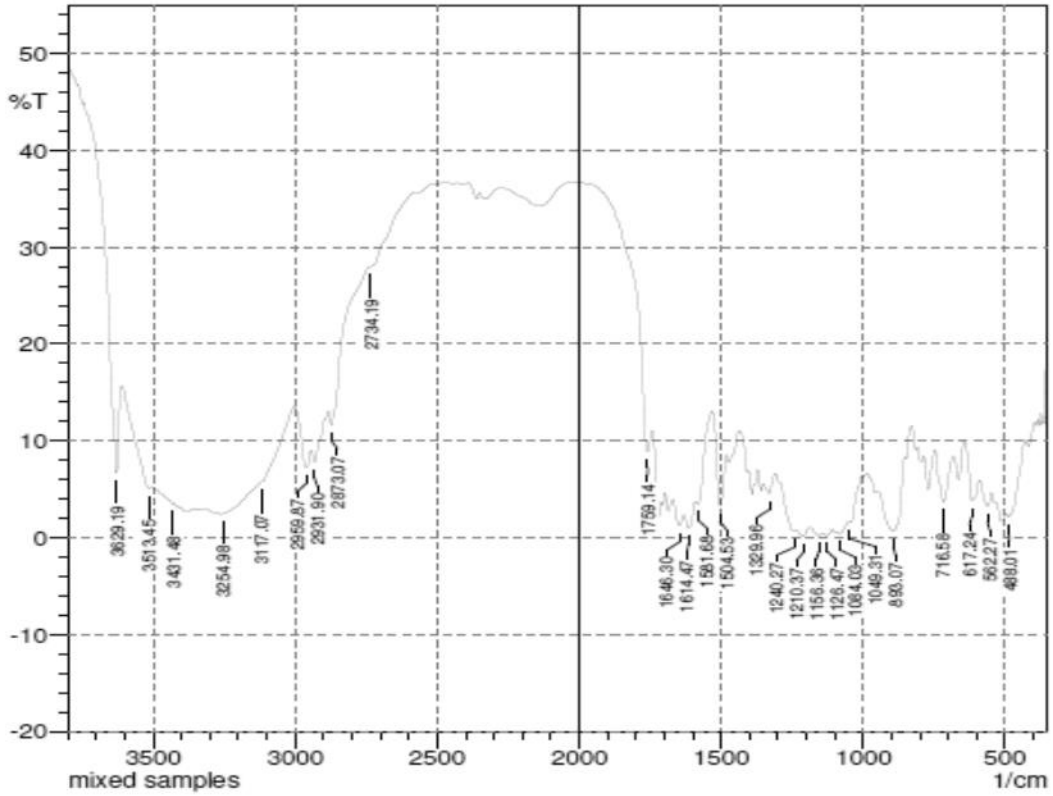


Table 16: FT-IR DATA OF PHYSICAL MIXTURE OF CAPECITABINE AND EXCIPIENTS

S. No	Wave Number cm^{-1}	Assignment
1	3196	O-H stretching
2	1556	C=O stretching
3	1722	C-F stretching
4	2910	C-H stretching

Drug – excipients compatibility was checked by comparing the IR spectra of pure drug, excipients and physical mixture of drug with excipients. No significant changes in the functional group between the spectra were observed. This ensured that there was no interaction between drug and excipients.

PREPARATION OF CAPECITABINE NANOPARTICLES:

Capecitabine nanoparticle was prepared by using chitosan as polymer, Tri polyphosphate as cross linking agent and calcium chloride as stabilizer along with solvent of water and acetic acid by ionic-gelation technique.

Figure 17: FORMATION OF NANOPARTICLE

PARTICLE SIZE AND ZETA POTENTIAL OF CAPECITABINE NANOFORMULATIONS:

Table 17

Formulation code	Particle size (nm)	PDI	Zeta potential (mV)
1	4028	0.617	60.1
2	1013	0.828	38.4
3	4057	0.257	60.7
4	967.4	0.994	36.2
5	1843	0.992	57.6
6	804.9	0.860	50.8
7	2665	1.000	52.7
8	773.2	0.937	39.5
9	616.3	0.702	34.2
10	474.4	0.730	19.5

Formulation 9, 10 was found to be 616.3 nm and 474.4 nm in particle size with zeta potential of 19.2 mV and 34.2 mV . So F-9, 10 was selected for further studies.

DETERMINATION OF PARTICLE SIZE AND ZETA POTENTIAL:

The average mean diameters and size distribution and the electrophoretic mobility (zeta potential) measurements of Capecitabine loaded nanoparticles was found out by photon correlation spectroscopy were made using Zeta sizer (Nano ZS90, Malvern Instruments) at 25˚C.

PARTICLE SIZE AND ZETA POTENTIAL FOR FORMULATION 9:

Figure 18: PARTICLE SIZE OF FORMULATION 9

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	616.3	Peak 1:	394.0	94.9	91.73
PdI:	0.702	Peak 2:	24.91	5.1	4.510
Intercept:	0.965	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

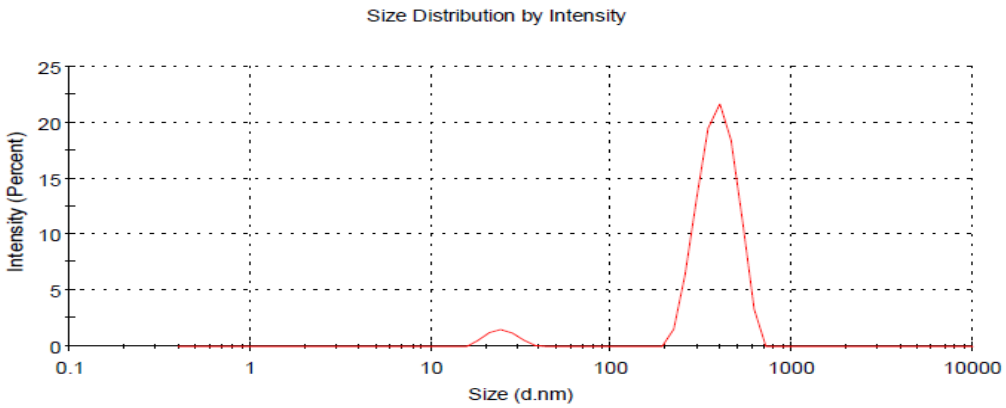
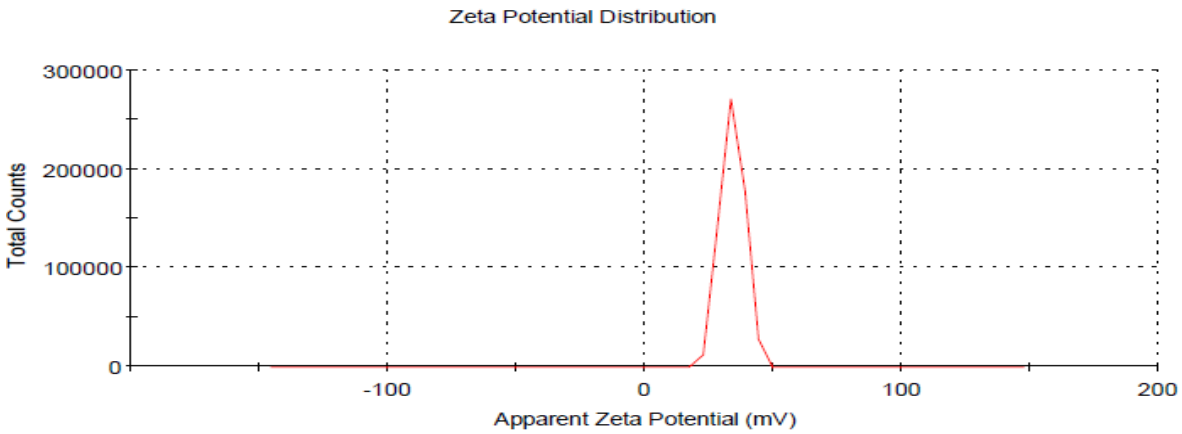


Figure 19: ZETA POTENTIAL OF FORMULATION 9

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	34.2	Peak 1:	34.2	100.0	4.69
Zeta Deviation (mV):	4.69	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.732	Peak 3:	0.00	0.0	0.00

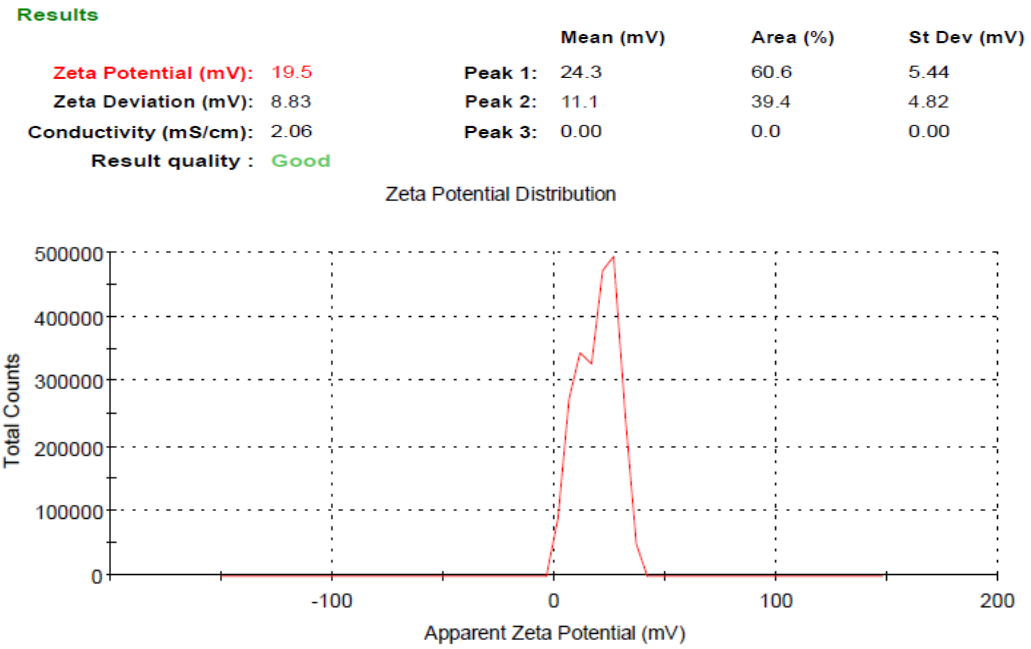
Result quality : Good



PATICLE SIZE AND ZETA POTENTIAL FOR FORMULATION 10:

Figure 20: PARTICLE SIZE OF FORMULATION 10

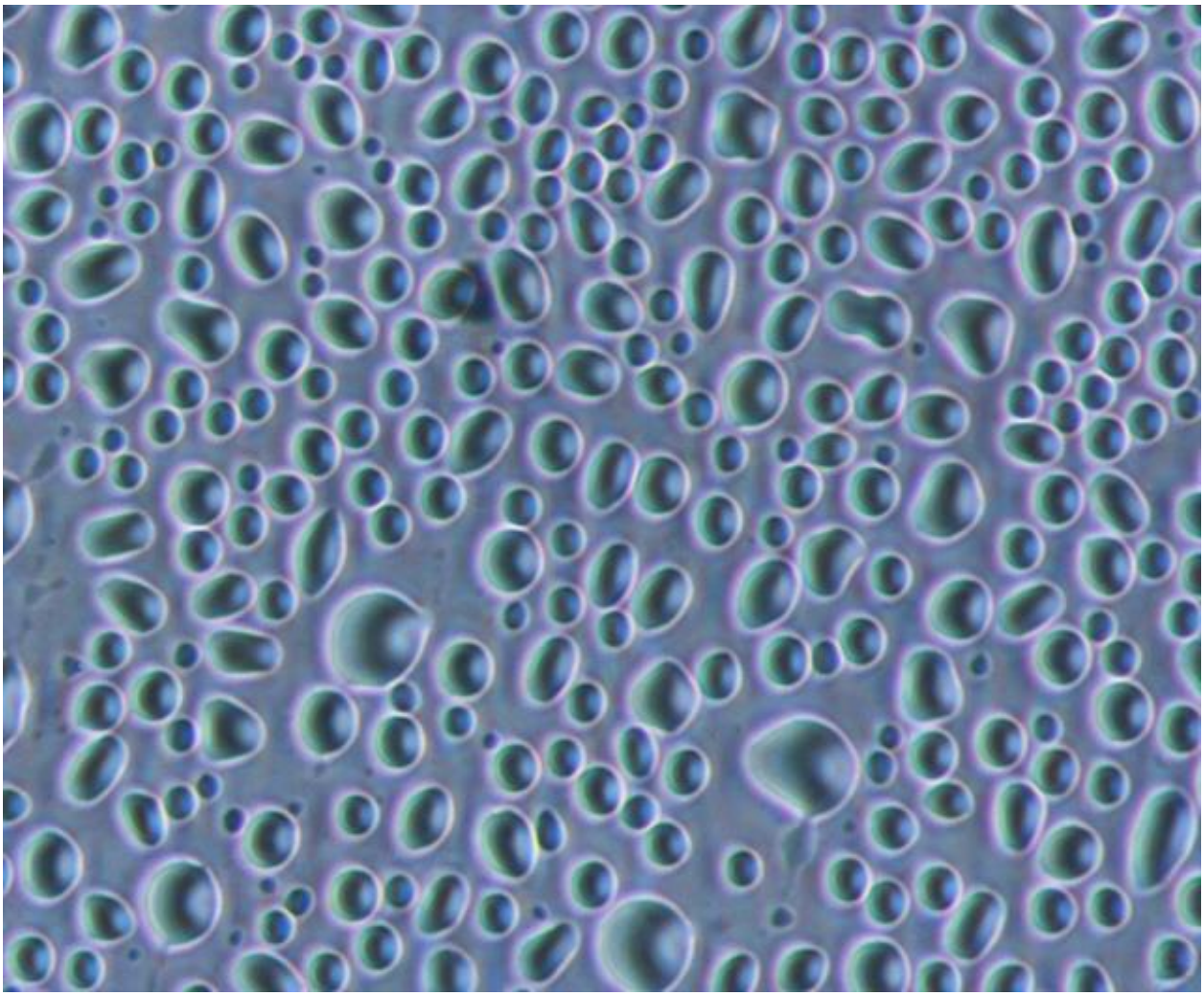
Figure 21: ZETA POTENTIAL OF FORMULATION 10



DETERMINATION OF MORPHOLOGY OF THE NANOSUSPENSION BY PHASE CONTRAST MICROSCOPY:

The drug loaded nanosuspension was observed under phase contrast microscopy to study the morphology of suspension.

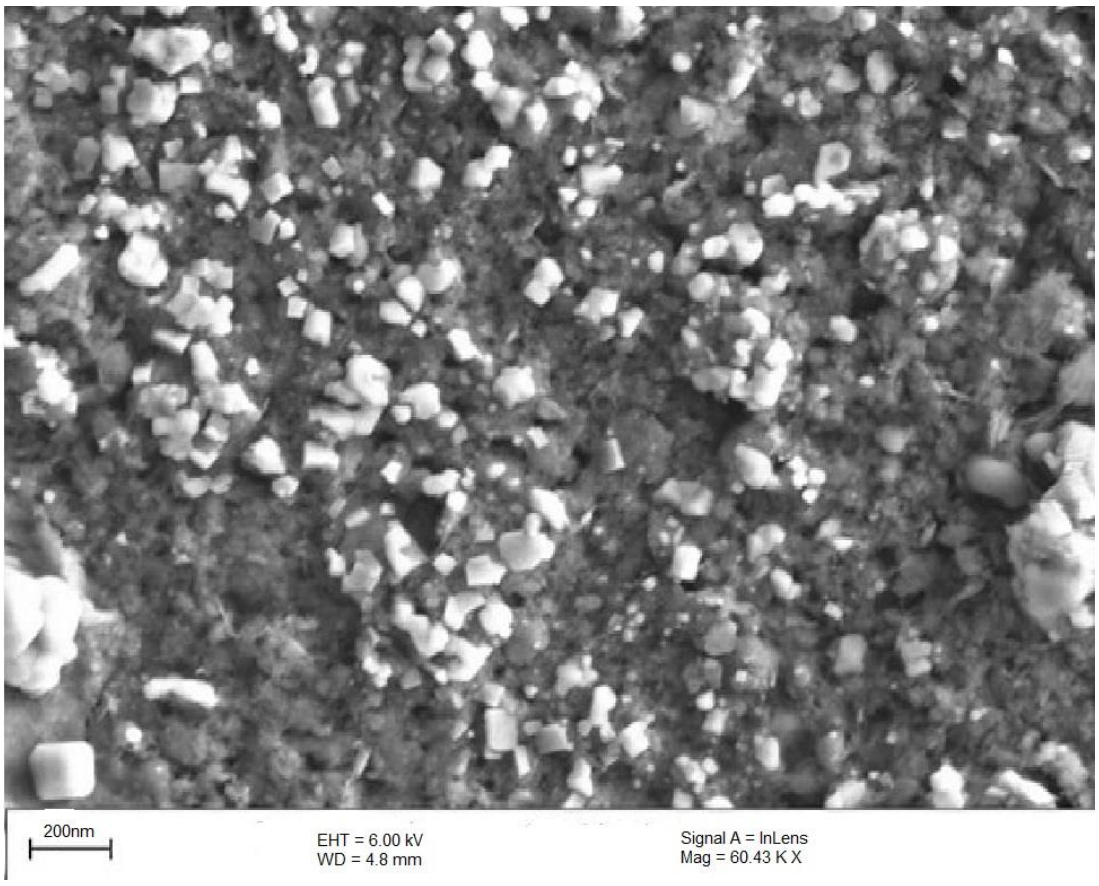
Figure 22: IMAGE OF PHASE CONTRAST MICROCOPY



SCANNING ELECTRON MICROSCOPY OF NANOFORMULATION OF CAPECITABINE:

The surface morphology and size for the selected suspensions was studied using field emission scanning electron microscopy (ΣIGMA FE-SEM; Zeiss Supra 40 apparatus Germini column, Germany) operated at 5 kV accelerating voltage. The images obtained showed cubic particles.

Figure 23: IMAGE OF SCANNING ELECTRON MICROSCOPY



FREEZE DRYING (LYOPHILIZATION):

Freeze drying was performed to convert the nanosuspension into powder. The selected formulations were kept in well closed containers and allowed to freeze under -80˚C for 4 hours. Then the formulations was freeze dried using LYODOL freeze dryer at -40˚C and -1.12 mbar pressure for a period of 72 hours. At the end of 72 hours a drug loaded white powder were obtained.

Figure 24: LYOPHILIZED CAPECITABINE NANOPARTICLES

DRUG CONTENT AND ENTRAPMENT EFFICIENCY OF THE FORMULATION:

DRUG CONTENT:

Amount of drug present in the lyophilized product was found to be 1.3 mg for formulation 9 and 5.4 mg for formulation 10, when compare to theoretical yield of 5.11 mg it was found that formulation 9 was having only 25.44% and formulation 10 was having 105.67%. Therefore formulation 10 was selected for further studies.

ENTRAPMENT EFFICIENCY:

Entrapment efficiency of the desired formulation 10 was determined.

Table 18: ENTRAPMENT EFFICIENCY OF THE FORMULATION

Formulation	Absorbance	Concentration(mcg/ml)	Amount of drug	Entrapment efficiency (%)
10	0.101	3	0.03	96.35±5

PREPARATION OF TABLETS:

Table 19: FORMULA FOR TABLETS

Ingredients	Amount per tablet (mg)
Nanoformulation	645
Magnesium stearate (2%)	12.9
Talc (1%)	6.45
Total	664.35

Figure 25: IMAGE OF TABLET

EVALUATION OF TABLETS:

PRE COMPRESSION EVALUATION:

Table 20: DATA OF ANGLE OF REPOSE

Product	r (cm)	h (cm)	$\Theta = \tan^{-1}h/r$	Flow property
Without excipients	1.5	2	53.12˚	Poor
With excipients	2.6	2	34.56˚	Good

Table 21: BULK AND TAPPED DENSITY, CARR'S INDEX AND HAUSNER'S RATIO OF FORMULATION

	Bulk density	Tapped density	Carr's index (%)	Hausner's ratio
Trial 1	0.5	0.58	13.79	1.16
Trial 2	0.5	0.57	12.28	1.14
Trial 3	0.42	0.48	12.47	1.14
Average	0.47±0.046	0.54±0.055	12.84±0.822	1.146±0.011

The bulk density and tapped density ranged from 0.5 and 0.58 respectively. The angle of repose and compressibility index (%) ranged from 34.56˚ and 13.79%. According to the guideline the angle of repose, compressibility index and hausner's ratio indicates good flow properties if the value of angle of repose (31-35) compressibility index (11-15) and hausner's ratio (1.12-1.18) of the powder mixture. These results show that the powder mixture has good flow properties.

POST COMPRESSION EVALUATION:

WEIGHT VARIATION TEST FOR FORMULATION 10:

Table 22: WEIGHT VARIATION DATA OF TABLETS

S. No	Weight of tablet (mg)
1	658
2	657.5
3	661
4	659
5	662
6	661.5
7	657
8	658
9	656
10	662
11	660
12	659
13	667.5
14	663
15	664
16	662.5
17	664
18	670
19	659
20	650
Average weight	660.5 ± 5%

Weight variation test was performed for 20 tablets. All the tablets passed the weight variation test as per the pharmacopoeial limit and was within 5% as shown in the table above.

FRIABILITY TEST FOR FORMULATION 10:

Table 23: FRIABILITY TEST DATA OF TABLETS

Tablets	Weight of 10 tablets before the test(mg)	Weight of 10 tablets after the test(mg)	%Friability
1	658	657	0.15
2	657.5	656	0.15
3	661	658	0.3
4	659	657	0.15
5	662	659	0.45
6	661.5	659	0.3
7	657	655	0.3
8	658	655	0.15
9	656	653	0.4
10	662	658	0.36
			Average weight=0.271

Friability test for tablets was carried out and it was passed the friability test as per the pharmacopoeial limit and was within 1% as shown in the table.

HARDNESS TEST FOR FORMULATION 10:

Table 24: HARDNESS TEST DATA OF TABLETS

Trial	Hardness (kg/cm^2)
Trial 1	5.98
Trial 2	5.95
Trial 3	6.00
Average	5.97 ± 0.025166

The hardness test was performed for the tablets. Then the values were within the limits prescribed in the pharmacopoeia as shown in the above table.

THICKNESS TEST FOR FORMULATION 10:

Table 25: THICKNESS TEST DATA OF TABLETS

Trial	Thickness (cm)
Trial 1	0.71
Trial 2	0.68
Trial 3	0.61
Average	0.66 ± 0.051316

The thickness test was performed for the tablets and the values were within the limit.

DISINTEGRATION TEST FOR FORMULATION 10:

Disintegration test was performed for the tablets from formulation using pH 1.2 and pH 6.8 as the medium. As the immersion fluid of pH 1.2 the tablets show no evidence of disintegration because of the enteric coating of the tablet. Then using the pH 6.8, tablets disintegrates within 30 mins.

***In vitro* DRUG RELEASE FOR FORMULATION 10:**

Table 26: RELEASE PROFILE OF FORMULATION

pH	Time(mins)	Absorbance	Concentration (mcg/ml)	Amount of drug release (mg)	% drug release (%)	Cumulative drug release (%)
1.2	60	0	-	-	-	-
	120	0	-	-	-	-
7.4	180	0.027	0.2	1.8	1.2	1.2
	240	0.046	0.5	4.5	3	3
	300	0.073	0.8	7.2	4.8	4.8
6.8	305	0.091	1	9	6	10.8
	310	0.153	1.7	15.3	10.2	15
	315	0.245	2.6	23.4	15.6	20.4
	330	0.398	4.4	39.6	26.4	31.2
	345	0.474	5.3	47.7	31.8	36.6
	360	0.590	6.6	59.4	39.6	44.4
	420	0.752	8.5	76.5	51	55.8
	480	0.827	9.1	81.9	54.6	59.4

In vitro drug release was performed and calculated. It shows the drug release in three different pH buffer solutions. In pH 1.2 (0.1N Hcl) the drug was not released because of the enteric coating layer of the tablet and in pH 7.4 phosphate buffer it shows the release of 4.8% at 3 hours. Then in pH 6.8 phosphate buffer the release was 54.6% at 3 hours. Total release was found to be 59.4% at 8 hours.

Figure 26: DISSOLUTION IMAGE OF FORMULATION

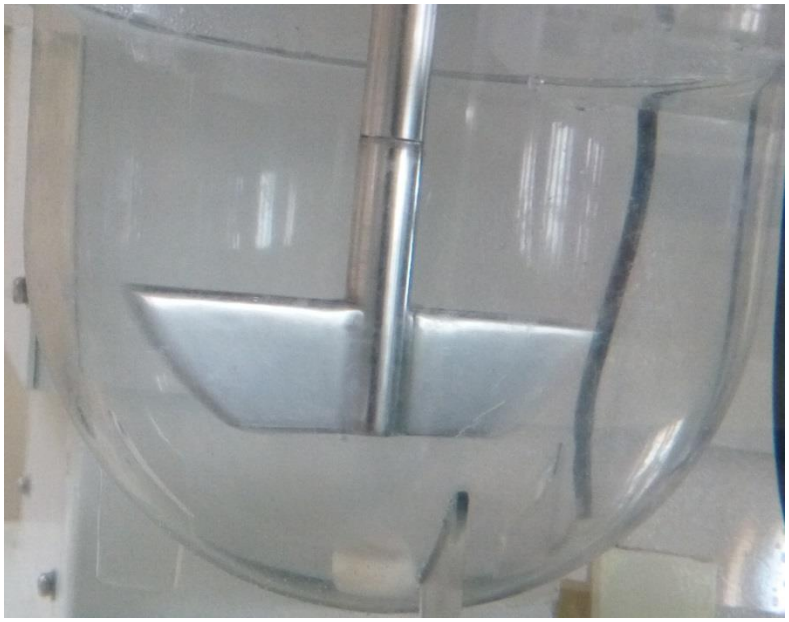
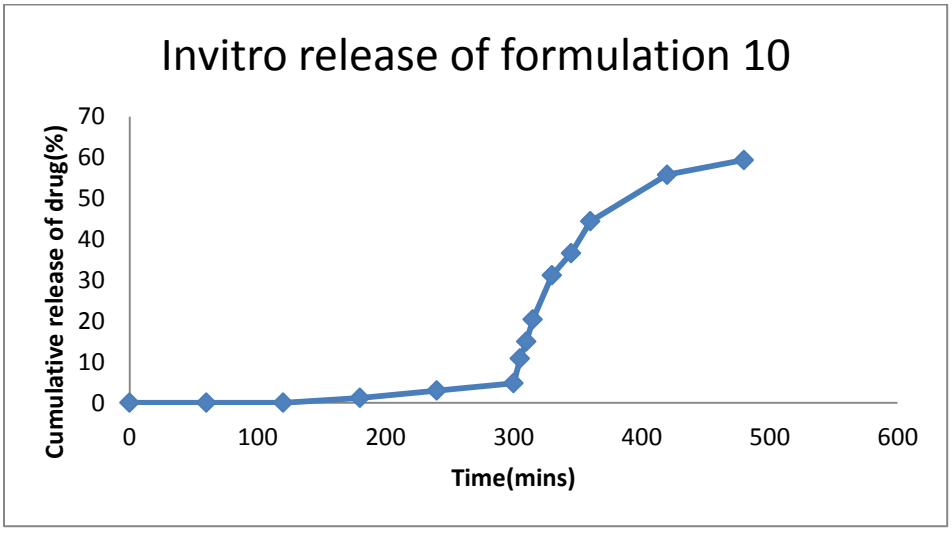


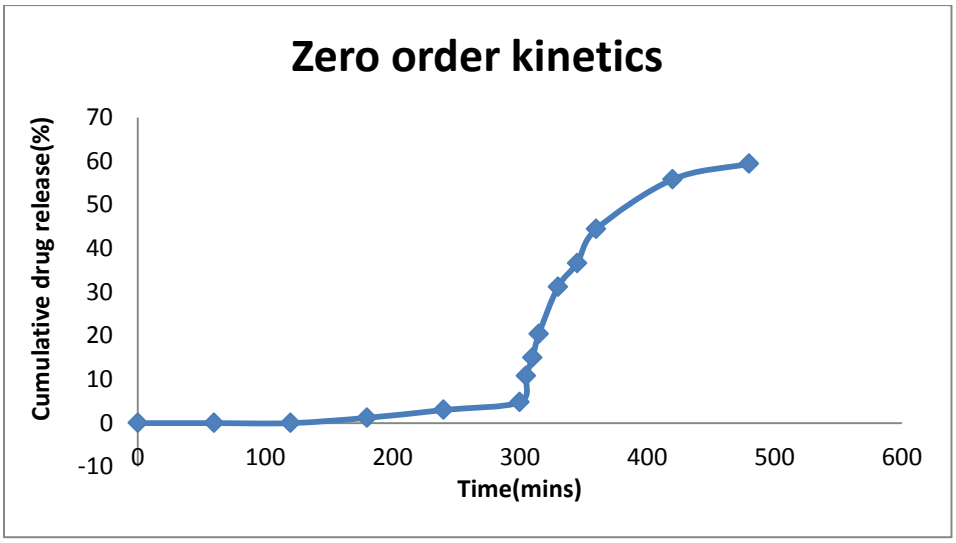
Figure 27: %DRUG RELEASE OF FORMULATION 10



DRUG RELEASE KINETICS:

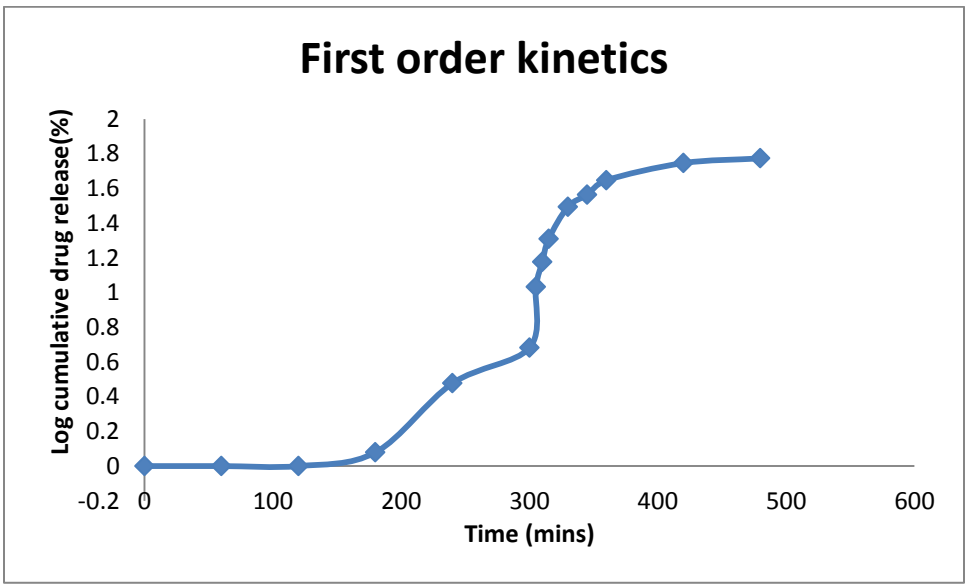
ZERO ORDER KINETICS OF FORMULATION 10:

Figure 28: ZERO ORDER RELEASE



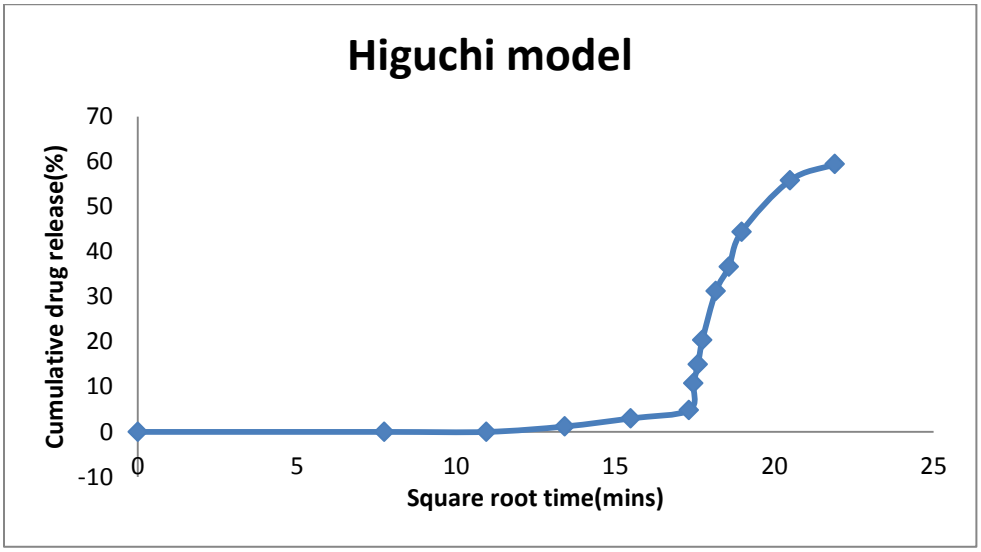
FIRST ORDER KINETICS OF FORMULATION 10:

Figure 29: FIRST ORDER RELEASE



HIGUCHI RELEASE OF FORMULATION 10:

Figure 30: HIGUCHI RELEASE



KORSMEYER PEPPAS OF FORMULATION 10:

Figure 31: KORSMEYER PEPPAS RELEASE

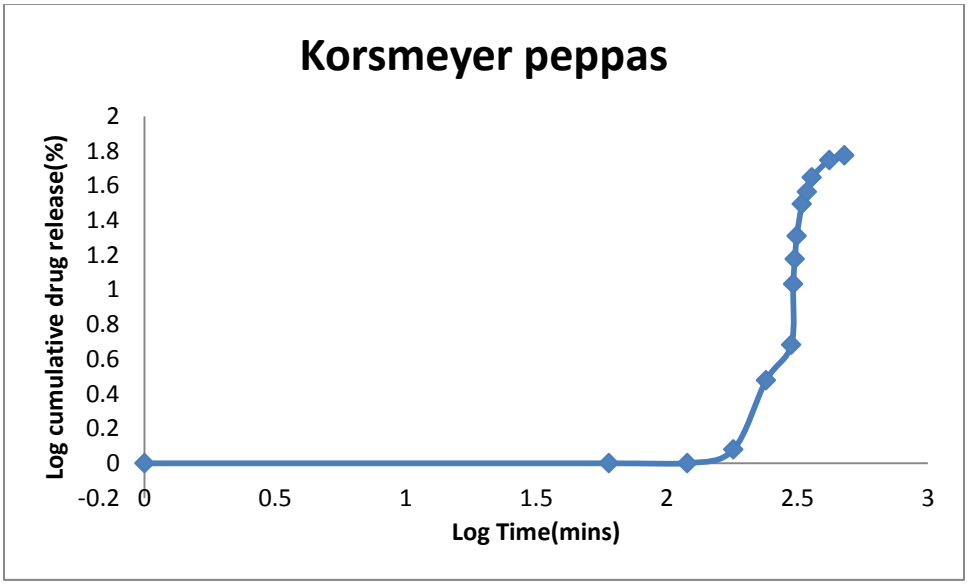


Table 27: RELEASE KINETICS DATA

Release kinetics model	R^2 values
Zero order kinetics	0.5909
First order kinetics	0.5385
Higuchi	0.3848
Korsmeyer peppas	0.8620 n = 2.762

Comparing the R^2 values, korsmeyer peppas gives high R^2 value. So, this korsmeyer peppas kinetics model considered as the best release kinetics. If n>1, this model is considered as the super case II transport.

CELL LINE STUDIES:

MTT ASSAY:

Cytotoxic activity of samples (µg/ml) IC_{50}

Table 28: DATA OF INHIBITORY CONCENTRATION

Sample	HT-29 (colon carcinoma)
Drug	46±1.5
Formulation	28±0.5
Standard : Doxorubicin	22±0.5

IC_{50} – Values of respective samples (at 24 hours)

$$\text{Formula: \% of viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

%CELL VIABILITY OF CAPECITABINE DRUG SOLUTION AND FORMULATION:

Figure 32: % CELL VIABILITY OF CAPECITABINE DRUG SOLUTION

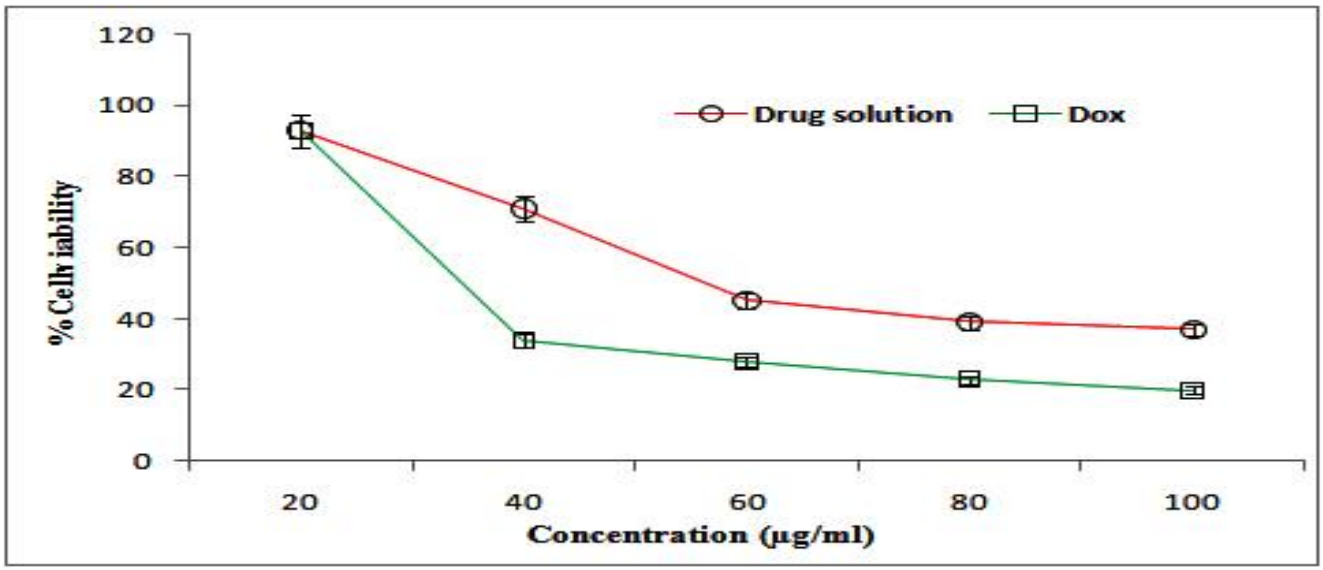
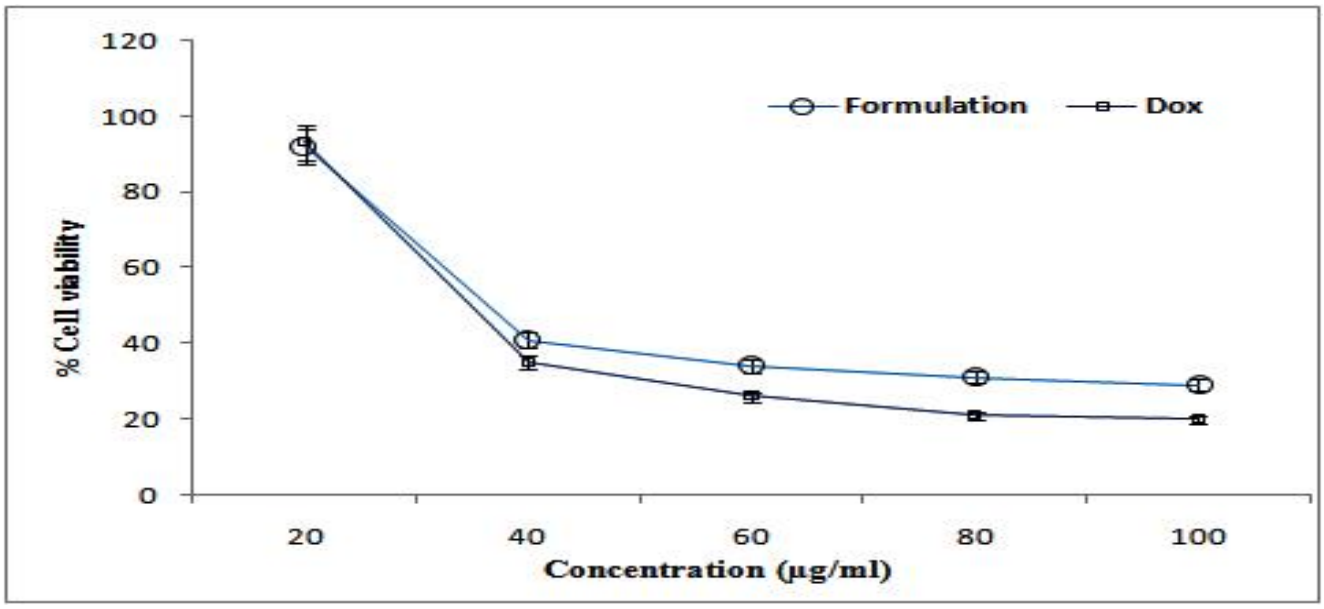


Figure 33: % CELL VIABILITY OF FORMULATION 10:



PHASE CONTRAST MICROSCOPY OF CAPECITABINE DRUG SOLUTION AND FORMULATION:

Figure 34: PCM OF CAPECITABINE DRUG SOLUTION

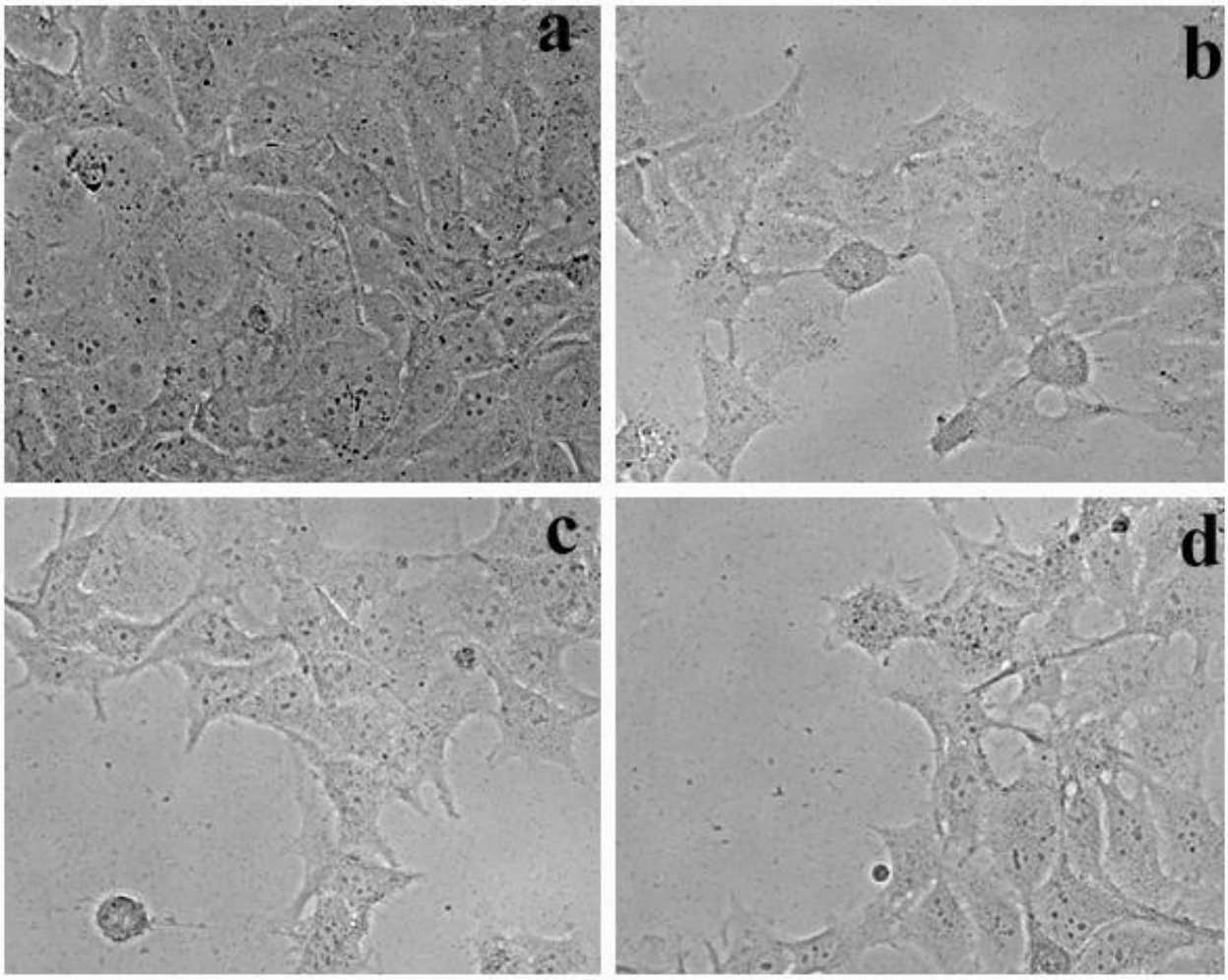
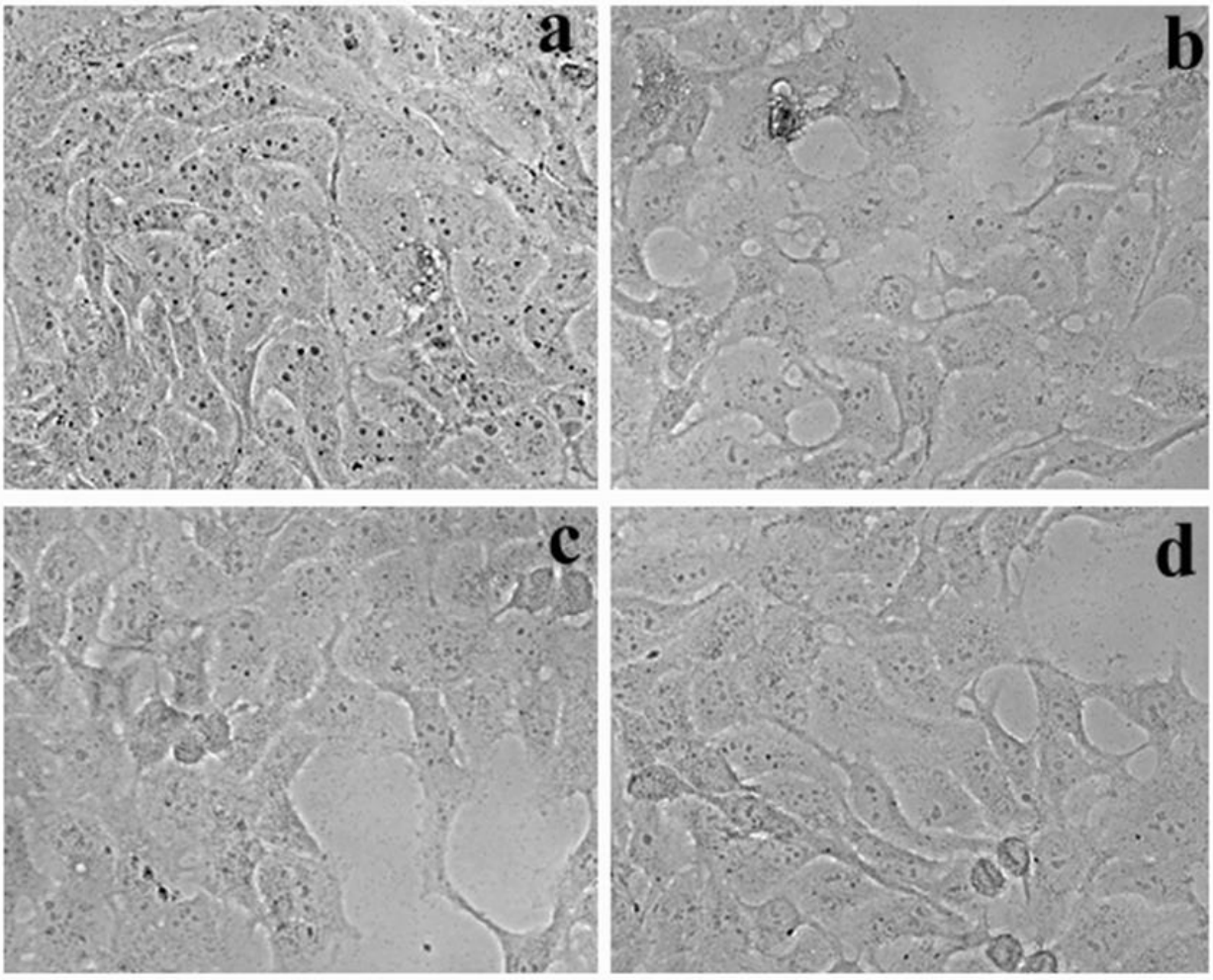


Figure 35: PCM OF FORMULATION 10



FLUORESCENCE MICROSCOPY OF CAPECITABINE DRUG SOLUTION AND FORMULATION:

Figure 36: FLUORESCENCE MICROSCOPY OF CAPECITABINE DRUG SOLUTION

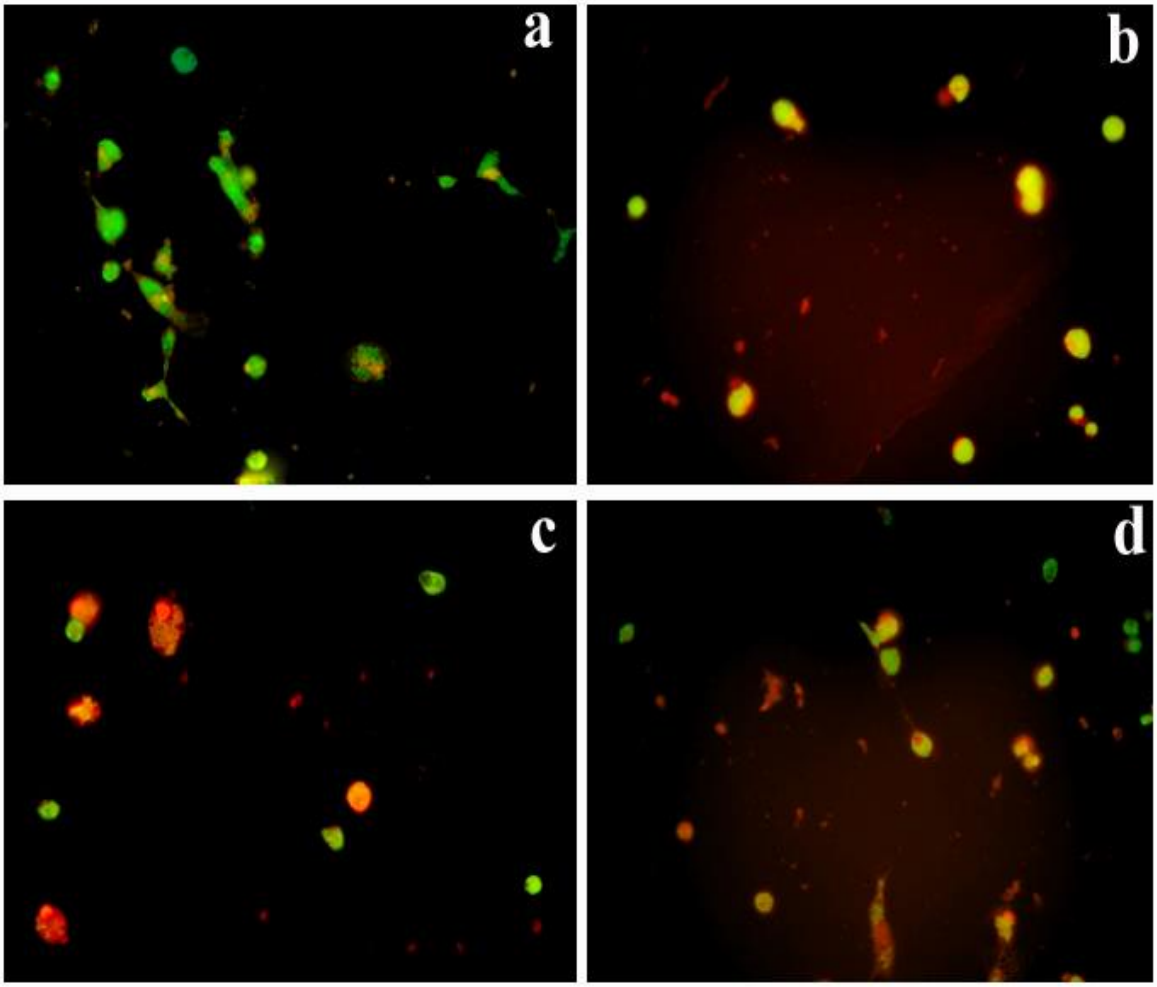
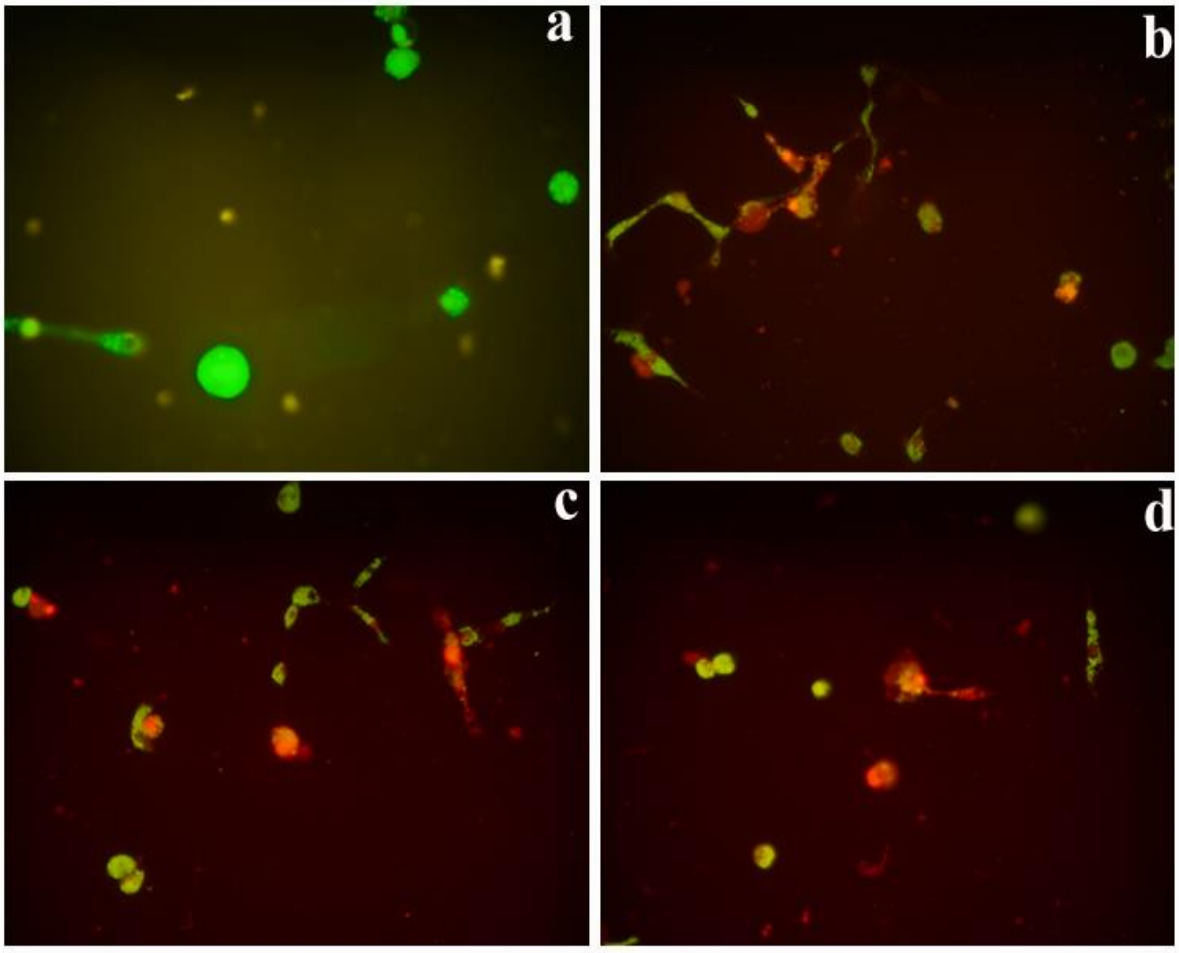


Figure 36: FLUORESCENCE MICROSCOPY OF FORMULATION 10



The inhibitory concentration (IC_{50}) means the concentration required to reduce the cell viability. IC_{50} of the capecitabine drug solution and formulation was found to be 46 µg/ml and 28 µg/ml respectively. The standard shows the inhibitory concentration of 22 µg/ml. Based on IC_{50}, the formulation showed better cytotoxic activity with decreased cell viability when compared with capecitabine drug solution. Cell viability was reduced on increasing concentration of formulation and capecitabine drug solution. When compared with control, formulation 10 shows better cytotoxic activity than capecitabine drug solution. This was assessed by phase contrast microscopy and fluorescence microscopy.

CONCLUSION

The study concludes that the drug capecitabine in nanoparticulate drug delivery system of ionic gelation technique yields 474.4 nm nanosize of particles with good zeta potential and SEM analysis also confirms the surface morphology of nanoparticles with cubic shape.

The prepared capecitabine nanoparticles were taken along with magnesium stearate and talc was taken as excipients for preparation of tablets by direct compression method. Evaluation of tablets such as weight variation test, Hardness test, Thickness test, Friability test and disintegration test was carried out and shown that the tablets were within the limit.

In -vitro dissolution study was performed for tablet and the release showed 59.4% at end of the 8 hours to target the colon. For release data, drug release kinetics was performed such as zero order kinetics, first order kinetics, Higuchi release and Korsmeyer peppas model.

Further, cell line study was carried out using HT 29 (Colon carcinoma) cells. This study confirmed, the formulation showed better cytotoxic activity than capecitabine drug solution.

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Certificates

Acknowledgement

Content

List of Tables

List of Figures

Abbreviations

Introduction

Literature Review

Aim and objective

Plan of Work

Drug Profile

Polymer profile

Excipient Profile

Materials & Equipments

Methodology

Preformulation Study

Result & Discussion

Conclusion

Bibliography

Annexure

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'Approach of Green Chemistry in Pharma, Chemical and Material Science Sectors - A perspective'

30th & 31st October 2015

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From

Dr. M.PaulPandi,
Department of zoology,
Bharathiar university,
Coimbatore-46,
Tamilnadu, India.

To

G. Suganya,
2nd year M.Pharm,
Department of Pharmaceutics,
PSG College of Pharmacy,
Coimbatore.

Dear student

The 2 samples [Drug solution and Formulation) given by G. Suganya, , were received and subjected to Cytotoxicity assay and Fluorescence microscopic assay on HT-29 cells (Colon carcinoma).

The results of the studies were verified and given with proper information through photographs and graphs.

30/8/17

[Signature with seal]

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