CHARACTERIZATION AND IN VITRO DRUG RELEASE PERFORMANCE OF EUDRAGIT COATED HIBISCUS ESCULENTUS-SODIUM ALGINATE BEADS FOR COLON SPECIFIC DELIVERY SYSTEM

A Dissertation Submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

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DECLARATION

I hereby declare that the thesis entitled **"CHARACTERIZATION AND IN VITRO DRUG RELEASE PERFORMANCE OF EUDRAGIT COATED HIBISCUS ESCULENTUS-SODIUM ALGINATE BEADS FOR COLON SPECIFIC DELIVERY SYSTEM"** has been originally carried out by me under the supervision and guidance of **Mrs. PRIYANKA SINHA, M.Pharm.,** Assistant Professor, Department of Pharmaceutics, C.L.Baid Metha College of Pharmacy, Chennai-97 during the academic year 2016-2017.

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INTRODUCTION

Oral delivery of drugs is by far the most preferred route of drug delivery due to ease of administration, patient compliance and flexibility in formulation.Conventional oral dosage forms provide a specific drug concentration in systemic circulation without offering any control over drug delivery[(1)].These systems achieve as well as maintain drug concentration within therapeutically effective range needed for treatment only when taken several times a day. This results in significant fluctuation in drug levels.Now-a-days most of the pharmaceutical scientists are involved in developing an ideal drug delivery system (DDS). An ideal oral drug delivery system should steadily deliver a measurable and reproducible amount of drug to the target site over a prolonged period. Oral route has been the most popular and successfully used for sustained delivery of drugs because of convenience and ease of administration, greater flexibility in dosage form design and ease of production and low cost of such a system. [(2)]

Controlled Drug Delivery Systems:

Controlled drug delivery is one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. Continuous oral delivery of drugs at predictable and reproducible kinetics for pre determined period throughout the course of GIT. Recently, a new generation of pharmaceutical products, called controlled release drug delivery systems, such as those developed from the osmotic pressure activated drug delivery system, have recently received regulatory approval for marketing, and their pharmaceutical superiority and clinical benefits over the sustained release and immediate release pharmaceutical products have been increased [(3)].

Controlled release drug administration means not only prolongation of the duration of drug delivery, similar to the objective in sustained release and prolonged release, but the term also implies the predictability and reproducibility of drug release kinetics. The basic rationale of controlled drug delivery system is to optimize the biopharmaceutical, pharmacokinetics and pharmacodynamic properties of drug in such a way that its utility is maximized through reduction in the side effects and cure or control of condition in the shortest possible time by the most suitable route.

Controlled release denotes that the system is able to provide some actual therapeutic control, whether this is of a temporal nature, spatial nature, or both. Controlled drug delivery occurs when a polymer is combined with a drug or active agent such that the release from the bulk material is pre-designed.

Controlled drug delivery systems have been developed which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and/or targeting the delivery of drug to a tissue.

Controlled drug delivery or modified drug delivery systems are conveniently divided into four categories:

- Delayed release
- Sustained release
- Site-specific targeting
- Receptor targeting

More precisely, Controlled delivery can be defined as [4].

1. Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects.
2. Localized drug action by spatial placement of a controlled release system adjacent to or in the diseased tissue.
3. Targeted drug action by using carriers or chemical derivatives to deliver drug to a particular target cell type.
4. Provide a physiologically/therapeutically based drug release system. In other words, the amount and the rate of drug release are determined by the physiological/ therapeutic needs of the body.

A controlled drug delivery system is usually designed to deliver the drug at particular rate. Safe and effective blood levels are maintained for a period as long as the system continues to deliver the drug. Controlled drug delivery usually results in substantially constant blood levels of the active ingredient as compared to the uncontrolled fluctuations observed when multiple doses of quick releasing conventional dosage forms are administered to a patient.

Advantages of Controlled Drug Delivery System: [5]

- Avoid patient compliance problems.
- Dosage frequency was reduced.
- Minimize or eliminate local side effects.
- Minimize or eliminate systemic side effects.
- Obtain less potentiating or reduction in drug activity with chronic use.
- Minimize drug accumulation with chronic dosing.
- Improve efficiency in treatment.
- Cures or controls condition more promptly.
- Improves control of condition i.e., reduced fluctuation in drug level.
- Improves bioavailability of some drugs.
- Make use of special effects, eg., Sustained-release aspirin for morning relief of arthritis by dosing before bedtime.

Economy i.e. reduction in health care costs. The average cost of treatment over an extended time period may be less, with less frequency of dosing, enhanced therapeutic benefits and reduced side effects. The time required for health care personnel to dispense and administer the drug and monitor patient is also reduced.

Disadvantages of Controlled Drug Delivery System:

Decreased systemic availability in comparison to conventional dosage forms, which may be due to incomplete release, increased first-pass metabolism, increased instability, insufficient residence time for complete release, site specific absorption, pH dependent stability etc.Poor in vitro – in vivo correlation.

Possibility of dose dumping due to food, physiologic or formulation variables or chewing or grinding of oral formulations by the patient and thus, increased risk of toxicity.

Retrievals of drug are difficult in case of toxicity, poisoning or hypersensitivity reactions.

Reduced potential for dosage adjustment of drugs normally administered in varying strengths.

Oral Controlled Drug Delivery Systems:

Oral controlled release drug delivery is a drug delivery system that provides the continuous oral delivery of drugs at predictable and reproducible kinetics for a predetermined

period throughout the course of GI transit and also the system that target the delivery of a drug to a specific region within the GI tract for either local or systemic action.

All the pharmaceutical products formulated for systemic delivery via the oral route of administration, irrespective of the mode of delivery (immediate, sustained or controlled release) and the design of dosage form (solid dispersion or liquid), must be developed within the intrinsic characteristics of GI physiology. Therefore the scientific framework required for the successful development of oral drug delivery systems consists of basic understanding of

- Physicochemical, pharmacokinetic and pharmacodynamic characteristics of the drug.
- The anatomic and physiologic characteristics of the gastrointestinal tract.

Physicochemical characteristics and the drug delivery mode of the dosage form to be designed.

The main areas of potential challenge in the development of oral controlled drug delivery systems are: -

- Development of a drug delivery system: To develop a viable oral controlled release drug delivery system capable of delivering a drug at a therapeutically effective rate to a desirable site for duration required for optimal treatment.
- Modulation of gastrointestinal transit time: To modulate the GI transit time so that the drug delivery system developed can be transported to a target site or to the vicinity of an absorption site and reside there for a prolonged period of time to maximize the delivery of a drug dose.
- Minimization of hepatic first pass elimination: If the drug to be delivered is subjected to extensive hepatic first-pass elimination, preventive measures should be devised to either bypass or minimize the extent of hepatic metabolic effect.

Conventional oral controlled dosage forms suffer from mainly two adversities. The short gastric retention time (GRT) and unpredictable gastric emptying time (GET). A relatively brief GI transit time of most drug products impedes the formulation of single daily dosage forms.

Altering the gastric emptying can overwhelm these problems. Therefore it is desirable, to formulate a controlled release dosage form that gives an extended GI residence time.

- Extended release dosage form with prolonged residence time in stomach are highly desirable for drugs.
- Those are locally active in stomach.
- Those have an absorption window in the stomach or in the upper small intestine.
- Those are unstable in the intestinal or colonic environment.
- Have low solubility at high pH values.

Extended Drug Delivery System:

Extended release formulation is an important program for new drug research and development to meet several unmet clinical needs. There are several reasons for attractiveness of these dosage forms provides increase bioavailability of drug product, reduction in the frequency of administration to prolong duration of effective blood levels, reduces the fluctuation of peak trough concentration and side effects and possibly improves the specific distribution of the drug [(6)].

Extended release drug delivery system achieves a slow release of the drug over an extended period of time or the drug is absorbed over a longer period of time. Extended release dosage form initially releases an adequate amount of drug to bring about the necessary blood concentration (loading dose, DL) for the desired therapeutic response and therefore, further amount of drug is released at a controlled rate (maintenance dose, DM) to maintain the said blood levels for some desirable period of time [(7)].

Oral extended release drug delivery system:

The oral extended release dosage forms offer the opportunity to provide constant or nearly constant drug plasma levels over an extended period of time following administration. Extended release DDS include single-unit, such as tablets or capsules, and multiple-unit dosage forms, such as mini tablets, pellets, beads or granules, either as coated (reservoir) or matrix devices.

Objectives of Extended Release Drug Delivery System:

Every novel drug delivery system had a rationale for developing the dosage form likewise, ERDDS also having some objectives that are discussed below:

Suitable Drug Candidate for Extended Release Drug Delivery System:

The drugs that have to be formulated as an ERDDS should meet following parameters.

- It should be orally effective and stable in GIT medium.
- Drugs that have short half-life, ideally a drug with half-life in the range of 2 – 4hrsmakes a good candidate for formulation into ER dosage forms Eg. Captopril, Salbutamol Sulphate.
- The dose of the drug should be less than 0.5g as the oral route is suitable for drugs given in dose as high as 1.0g Eg. Metronidazole.
- Therapeutic range of the drug must be high. A drug for ERDDS should have therapeutic range wide enough such that variations in the release do not result in concentration beyond the minimum toxic levels14.

Factors Affecting the Extended Release Drug Delivery System: [7]

Physiochemical Properties:

Aqueous Solubility:

Certain drug substance having low solubility is reported to be 0.1 mg/ml. As the drug must be in solution form before absorption, drug having low aqueous solubility usually suffers oral bioavailability problem due to limited GI transit time of un dissolved drug and limited solubility at absorption site. So these types of drug are undesirable to be formulated as extended release drug delivery system. Drug having extreme aqueous solubility are undesirable for extended release because, it is too difficult to control release of drug from the dosage form.

Partition Co-efficient:

As biological membrane is lipophilic in nature through which the drug has topass, so partition co-efficient of drug influence the bioavailability of drug very much. Drug having lower partition co-efficient values less than the optimum activity are undesirable for oral ER drug delivery system, as it will have very less lipid solubility and the drug will be localized at the first aqueous phase it come in contact. Drughaving higher partition co-efficient value

greater than the optimum activity are undesirable for oral ER drug delivery system because more lipid soluble drug will not partition out of the lipid membrane once it gets in the membrane.

Protein Binding:

The Pharmacological response of drug depends on unbound drug concentration rather than total concentration and all drugs bound to some extent to plasma or tissue proteins. Proteins binding of drug play a significant role in its therapeutic effect regardless the type of dosage form as extensive binding to plasma increase biological half-life and thus, such type of drug will release up to extended period of time then there is no need to develop extended release drug delivery for this type of drug.

Drug Stability:

As most of ER Drug delivery system is designing to release drug over the length of the GIT, hence drug should be stable in GI environment. So drug, which is unstable, can't be formulated as oral ER drug delivery system, because of bioavailability problem.

Mechanism and Site of Absorption:

Drug absorption by carrier mediated transport and those absorbed through a window are poor candidate for oral ER drug delivery system. Drugs absorbed by passive diffusion, pore transport and through over the entire length of GIT are suitable candidates for oral ER drug delivery system.

Dose size:

If a product has dose size > 0.5g it is a poor candidate for ER drug delivery system, because increase in bulk of the drug, thus increases the volume of the product. Thus dose of drug should small to make a good drug candidate for extended release drug delivery system.

Biological Properties:

Absorption:

The absorption behavior of a drug can affect its suitability as an extended release product. The aim of formulating an extended release product is to place a control on the delivery system. It is essential that the rate of release is much slower the rate of absorption. If we assume the transit time of most drugs and devices in the absorptive areas of GI tract is about 8-12 hours, the maximum half-life for absorption should be approximately 3-4 hours.

Otherwise the device will pass out of absorptive regions before drug release is complete. Therefore the compounds with lower absorption rate constants are poor candidates for extended release systems. Some possible reasons for a low extent of absorption are poor water solubility, small partition co-efficient, acid hydrolysis, and metabolism or its site of absorption.

Distribution:

The distribution of drugs in tissues can be important factor in the overall drug elimination kinetics. Since it not only lowers the concentration of circulating drug but it also can be rate limiting in its equilibrium with blood and extra vascular tissue, consequently apparent volume of distribution assumes different values depending on time course of drug disposition. Drugs with high apparent volume of distribution, which influence the rate of elimination of the drug, are poor candidate for oral ER drug delivery system e.g. Chloroquine. For design of extended release products, one must have information on disposition of the drug.

Metabolism:

Drug, which extensively metabolized is not suitable for ER drug delivery system. A drug capable of inducing metabolism, inhibiting metabolism, metabolized at the site of absorption or first-pass effect is poor candidate for ER delivery, since it could be difficult to maintain constant blood level e.g. levodopa, nitroglycerine. Drugs that are metabolized before absorption, either in lumen or the tissues of the intestine, can show decreased bioavailability from the extended releasing systems. Most intestinal walls are saturated with enzymes. As drug is released at a slow rate to these regions, lesser drug is available in the enzyme system. Hence the systems should be devised so that the drug remains in that environment to allow more complete conversion of the drug to its metabolite.

Half-life of Drug:

A drug having biological half-life between 2 to 8 hours is best suited for oral ER drug delivery system. As if biological half-life < 2hrs the system will require unacceptably large rate and large dose and biological half-life> 8hrs formulation of such drug into ER drug delivery system is unnecessary.

Pharmacokinetic parameters: [8]

Elimination half-life (t ½):

Drugs having a t ½ and 8 hours are ideally suited for CRDDS. If the t ½ is less than 1 hour the dose size required to be incorporated for a 12 hour or 24 hour duration dosage form may be too large. If the t ½ is very long there is usually no need for a CRDDS, unless it is simply intended for a reduction in fluctuation of steady state blood levels.

Total clearance (CL):

CL is a measure of the volume of distribution cleared of drug per unit of time. It is the key parameter in estimating the required dose rate for CRDDS, and predicting the steady state concentration.

Terminal disposition rate constant (Ke or λz):

The terminal disposition rate constant or elimination rate constant can be obtained from the t ½ and is required to predict a blood level time profile.

Apparent volume of distribution (V_d):

The Vd is the hypothetical volume of a drug would occupy if it were dissolved at the same concentration as that found in blood. It is the proportionality constant relating the amount of drug in the body to the measured concentration in the blood. Among the trio CL, Vd, and t½, the former two parameters are the independent variables and the last one is the dependent variable. The Vd or CL is required to predict the concentration time profile.

Absolute bioavailability (F):

The absolute bioavailability is the percentage of drug taken up into systemic circulation upon extra vascular administration. For drugs to be suitable for CRDDS one wants an F value to be close to 100%.

Intrinsic absorption rate constant (Ka):

The intrinsic absorption rate constant of the drug administered per oral in the form of a solution should be high, generally by an order of magnitude higher than the desired release rate constant of the drug from the dosage form, in order to insure that release process is the rate controlling step.

Therapeutic concentration (Css):

The therapeutic concentrations are the desired or target steady state peak concentrations (Css max), the desired or target steady state minimum concentrations (Css min), and the mean steady state concentration (Css.avg). The difference between Css max and Css min is the fluctuation. The smaller the desired fluctuation the greater must be the precision of the dosage form performance. The lower Css, the smaller Vd, the longer t½, the higher F and The less amount of drug is required to be incorporated into a CRDDS.

In vitro In vivo correlation: [9, 10]

In vitro-in vivo correlation plays a key role in the drug development and optimization of formulation. The in vitro release data of a dosage form containing the active substance serve as characteristic in vitro property, while the in vivo performance is generally represented by the time course of the plasma concentration of the active substance. These in vitro and in vivo data are then treated scientifically to determine correlations. For oral dosage forms, the in vitro release is usually measured and considered as dissolution rate. The relationship between the in vitro and in vivo characteristics can be expressed mathematically by a linear or nonlinear correlation. However, the plasma concentration cannot be directly correlated to the in vitro release rate; it has to be converted to the in vivo release or absorption data, either by pharmacokinetic compartment model analysis or by linear system analysis.

Advantages of Extended release dosage forms:

- The extended release formulations may maintain therapeutic concentrations over prolonged periods.
- The use of extended release formulations avoids the high blood concentration.
- Extended release formulations have the potential to improve the patient compliance.
- Reduce the toxicity by slowing drug absorption.
- Increase the stability by protecting the drug from hydrolysis or other derivative changes in gastrointestinal tract.
- Minimize the local and systemic side effects.
- Improvement in treatment efficacy.
- Minimize drug accumulation with chronic dosing.
- Usage of less total drug.

- Improvement the bioavailability of some drugs.
- Improvement of the ability to provide special effects.

Ex: Morning relief of arthritis through bed time dosing.

Disadvantages of Extended release dosage forms:

- High cost of preparation.
- The release rates are affected by various factors such as, food and the rate transit through the gut.
- Some differences in the release rate from one dose to another dose but these have been minimized by modern formulations.
- Extended release formulation contains a higher drug load and thus any loss of integrity of the release characteristics of the dosage form.
- The larger size of extended release products may cause difficulties in ingestion or transit through gut.

SUSTAINED DRUG DELIVERY SYSTEMS (SDDS)

Time release technology, also known as sustained – release, sustained – action, extended – release, time – release or timed – release, controlled – release, modified release, or continuous – release, is a mechanism used in pill tablets or capsules to dissolve slowly and release a drug over time. The advantages of sustained – release tablets or capsules are that they can often be taken less frequently than immediate - release formulations of the same drug, and that they keep steadier levels of the drug in the bloodstream [(11)].

Sustained release tablets and capsules are commonly taken only once or twice daily, compared with their counterpart, conventional forms that may have to be taken three or four times daily to achieve the same therapeutic effect. Typically, sustained release products provide an immediate release of drug that promptly produces the desired therapeutic effect, followed by gradual release of additional amounts of drug to maintain this effect over a predetermined period.

The basic rationale of a sustained drug delivery system is to optimize the biopharmaceutical, pharmacokinetic and pharmacodynamic properties of a drug in such a way that its utility is maximized through reduction in side effects and cure or control of condition in the shortest possible time by using smallest quantity of drug, administered by the

most suitable route. The goal of sustained drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration for a prolonged period of time. [12]

Advantages of sustained release dosage forms

a) Patient Compliance: Lack of compliance is generally observed with long term treatment of chronic disease, as success of drug therapy depends upon the ability of patient to comply with the regimen. The problem of lack of patient compliance can be resolved to some extent by administering SDDS.

b) Reduced 'see - saw' fluctuation: Administration of a drug in a conventional dosage form [except via intravenous infusion at a constant rate] often results in 'see – saw' pattern of drug concentration in the systemic circulation and tissue compartments depending on drug kinetics such as the rate of absorption, distribution, elimination and dosing intervals. A well designed sustained release drug delivery system can significantly reduce the frequency of drug dosing and also maintain a steadier drug concentration in blood circulation and target tissue cells.

c) Reduced total dose: SDDS have repeatedly been shown to use less amount of total drug to treat a diseased condition. By reducing the total amount of drug, decrease in systemic or local side effects are observed. This would also lead to greater economy.

d) Improved efficiency in treatment: Optimal therapy of a disease requires an efficient delivery of active drugs to the tissues, organs that need treatment. Very often doses far in excess to those required in the cells have to be administered in order to achieve the necessary therapeutically effective concentration. This leads to undesirable, toxicological and immunological effects in non-target tissue. A controlled release dosage forms leads to better management of the acute or chronic disease condition.

e) Economy: In comparison with conventional dosage forms the average cost of treatment over an extended period may be less. Economy also may result from a decrease in nursing time and hospitalization. Safety margin of high potency drugs can be increased.

f) Improved therapy:

- Sustained blood level: The dosage form provides uniform drug availability / blood levels unlike peaks and valley pattern obtained by intermittent administration.

- Attenuation of adverse effects: The incidence and intensity of undesirable effects caused by excessively high peak drug concentration resulting from the administration of conventional dosage forms is reduced.

- It is seldom that a dose is missed because of non-compliance by the patient.

Disadvantages of Sustained Release Dosage Forms

a) Dose dumping: Dose dumping is a phenomenon where by relatively large quantities of drug in a sustained release formulation is rapidly released, introducing potential toxic quantities of the drug into the systemic circulation. Dose dumping can lead to fatalities in case of potent drug, which have a narrow therapeutic index e.g. Phenobarbital

b) Less flexibility in accurate dose adjustment: In conventional dosage forms, dose adjustments are much simpler e.g. tablet can be divided into two fractions. In case of SDDS, this appears to be much more complicated. Controlled release property may get lost, if dosage form is fractured.

c) Poor in vitro – in vivo correlation: In SDDS, the rate of drug release is deliberately reduced to achieve drug release possibly over a large region of gastrointestinal tract. Here the so called 'Absorption window' becomes important and may give rise to unsatisfactory drug absorption in vivo despite excellent in-vitro release characteristics.

d) Patient variation: The time period required for absorption of drug released from the dosage form may vary among individuals. Co-administration of other drugs, presence or absence of food and residence time in gastrointestinal tract is different among patients. This also gives rise to variation in clinical response among the patient.

Designing of sustained release drug delivery system

Most of the orally administered drugs, targeting is not a primary concern and it is usually intended for drugs to penetrate to the general circulation and perfuse to other body tissues. For this reason, most systems employed are of the sustained release variety. It is

assumed that increasing concentration at the absorption site will increase circulating blood levels, which in turn, promotes greater concentration of drug at the site of action. If toxicity is not an issue, therapeutic levels can thus be extended.

These dosage forms must function in an environment of changing pH, the stomach being acidic and the small intestine more neutral, the effect of pH on the release process must be defined.

a) Partition Coefficient: The partition coefficient is an important drug property, which influences the design of oral SDDS as it governs the permeation of drug particles through biological membrane and diffusion of drug molecules across rate controlling membranes. Compounds which are lipophilic in nature having high partition coefficient are poorly aqueous soluble and retained in the lipophilic tissue for a longer time and vice versa. The choice of diffusion – limiting membranes must largely depend on the partitioning characteristics of the drug.

b) Drug stability: The stability of the drugs at the site of its release and exposure bio-milieu is one more drug property that can influence the design of oral controlled drug delivery. Drugs that are unstable in gastric pH can be developed as slow release dosage form and drug release can be delayed till the dosage form reaches the intestine. Drugs that undergo gut-wall metabolism and show instability in small intestine are not suitable for controlled drug delivery systems. Orally administered drugs can be a subject to both acid-base hydrolysis and enzymatic degradation. Degradation will proceed at a reduced rate for drugs in solid state; therefore, this is the preferred composition of delivery for problem cases.

c) Absorption: The rate, extent and uniformity of absorption of a drug are important factors when considering its formulation into a sustained – release system. Since the rate limiting step in drug delivery from a SDDS is its release from a dosage form, rather than absorption, a rapid rate of absorption of drug relative to its release is essential if system is to be successful. Since the purpose of forming a sustained release product is to place control on the delivery system, it is necessary that the rate of release is much slower than the rate of absorption.

d) Disease State: In some cases before the designing of an oral sustained delivery system for a drug it is important to consider the patient body condition and requirement.

Characteristics of drugs unsuitable for oral sustained release forms

a) Not effectively absorbed in the lower intestine e.g. riboflavin, ferrous salts
b) Absorbed and excreted rapidly; short biologic half-lives (< 1hr) e.g. penicillin G
c) Long biologic half-lives (>12 hr.) e.g. diazepam, phenytoin
d) Large doses required (>1g) e.g. Sulfonamides
e) Drugs with low therapeutic indices e.g. phenobarbital, digitoxin
f) Precise dosage titrated to individual is required e.g. anticoagulants, cardiac glycosides
g) No clear advantage for sustained release formulation e.g. Griseofulvin

Criteria for choosing drugs for sustained release dosage forms

a) Desirable half-life: The half-life of a drug is an index of its residence time in the body. If the drug has a short half-life (less than 2 hours), the dosage form may contain a prohibitively large quantity of the drug while drugs with elimination half-life of eight hours or more are sufficiently sustained in the body, when administered in conventional dosage from. SDDS is generally not necessary in such cases. Ideally, the drug should have half-life of three to four hours. Therapeutic compounds with short half-life are generally are excellent candidate for sustained-release formulations, as this can reduce dosing frequency.

b) High therapeutic index: Drugs with low therapeutic index are unsuitable for incorporation in controlled release formulations. If the system fails in the body, dose dumping may occur, leading to fatalities e.g. Digitoxin.

c) Small dose: If the dose of a drug in the conventional dosage form is high, its suitability as a candidate for sustained release is seriously undetermined. This is chiefly because the size of a unit dose sustained formulation will become too big to administer.. In general, a single dose of 0.5 - 1.0 g is considered maximal for a conventional dosage form. This also holds for sustained release dosage form. Another consideration is the margin of safety involved in administration of large amount of a drug with a narrow therapeutic range.

d) Desirable absorption and solubility characteristics: Absorption of poorly water soluble drug is often dissolution rate limited. Incorporating such compounds into controlled release formulations is therefore unrealistic and may reduce overall absorption efficiency.

e) Desirable absorption window: Certain drugs when administered orally are absorbed only from a specific part of gastrointestinal tract. This part is referred to as the 'absorption window'. Drugs exhibiting an absorption window like fluorouracil, thiazide diuretics, if formulated as sustained release dosage form are unsuitable.

f) First pass clearance: Delivery of the drug to the body in desired concentrations is seriously hampered in case of drugs undergoing extensive hepatic first pass metabolism, when administered in sustained release forms.

COLON TARGETING DRUG DEIVERY SYSTEMS (CTDDS)

Colonic delivery offers several potential therapeutic advantages as a site for drug delivery,

- The colon is rich in lymphoid tissue, uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery.
- The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability.
- The colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs.
- Reduced proteolytic activity in the colon may be helpful in achieving reasonable absorption of certain drugs that are enzymatically labile in small intestine.
- Reduced fluid motility and motility in the colon when compared with small intestine is advantageous formulation consists of multiple components such as permeation enhancers that must reach epithelial layer to achieve close spatial proximity with each other.
- The colonic region has somewhat less hostile environment with less diversity and less intensity of activity as compared to stomach and small intestine. [13-15]

Targeting of drugs to the colon is of increasing importance for local treatment of inflammatory bowel diseases (IBD) of the colon such as ulcerative colitis and crohn's disease (CD) and in treatment of colorectal cancers. Treatment might be more effective if the drug substances were targeted directly on the site of action in the colon. Lower doses might be adequate and, if so, systemic side effects might be reduced. A number of other serious diseases of the colon, e.g. colorectal cancer, might also be capable of being treated more effectively if drugs were targeted on the colon. Site-specific means of drug delivery could also allow oral administration of peptide and protein drugs, which normally become inactivated in the upper parts of the gastrointestinal tract. [16,17]

Colonic drug delivery can be achieved by oral or rectal administration. With regard to rectal route, the drugs do not always reach the specific sites of the colonic disease and the sites of colonic absorption. To reach the colon and to be able to specifically deliver and absorb the drug there, the dosage form must be formulated taking into account the obstacles of gastrointestinal tract. [18,19]

Factors to be considered in designing CTDDS

Formulations for colonic delivery are, in general, delayed-released dosage forms which may be designed either to provide a 'burst release' or a sustained / prolonged / targeted.

Factors to be considered for designing CTDDS are explained below:

(A) Anatomy and physiology of colon [20]

The large intestine has 3 parts: the cecum, the colon, and the rectum. The main function of the large intestine is to remove water and salts (electrolytes) from the undigested material and to form solid waste (feces) that can be excreted. The remaining contents of the large intestine move to the rectum, where feces are stored until they leave the body through the anus as a bowel movement.

In mammals colon is further subdivided into the ascending colon, transverse colon, the descending colon and the sigmoid colon. The colon from cecum to the mid transverse colon is also known as the right colon. The remainder is known as the left colon. The location of the parts of the colon is either in the abdominal cavity or behind it in the retro-peritoneum. The anatomy of colon is depicted by Fig. 1.

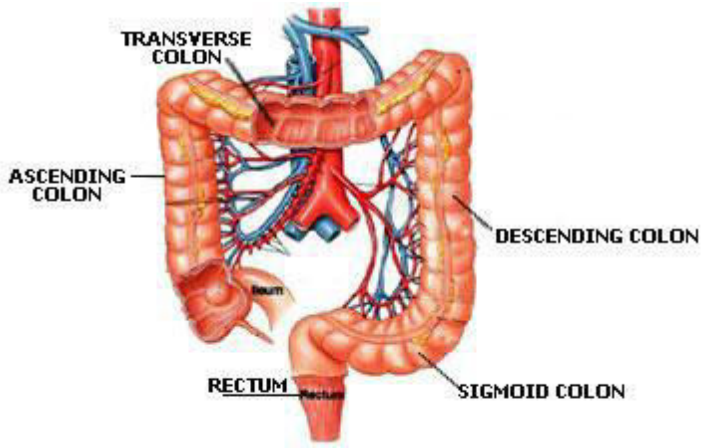

Figure: 1 Anatomy of Colon

- **Ascending colon:**

The ascending colon is on the right side of the abdomen. It is the part of the colon from the cecum to the hepatic flexure (the turn of the colon by the liver). It is retroperitoneal in most humans. In grazing animals the cecum empties into the spiral colon.

- **Transverse colon:**

The transverse colon is the part of the colon from the hepatic flexure to the splenic flexure. The transverse colon hangs off the stomach, attached to it by a wide band of tissue called the greater omentum. On the posterior side, the transverse colon is connected to the posterior abdominal wall by a mesentery known as the transverse mesocolon. As the path progresses from intestine the solid content increases as water gets absorbed.

- **Descending colon:**

The descending colon is the part of the colon from the splenic flexure to the beginning of the sigmoid colon. It is retroperitoneal in two-thirds of humans. In the other third, it has a (usually short) mesentery.

- **Sigmoid colon:**

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

(B) pH of the colon [20]

High pH gradient exists between the different parts of GIT. pH gradient between saliva and gastric juice and between gastric juice and intestinal juice is considerably high but that between different parts of intestine is low. The pH of the GIT is subject to both inter and intra subject variations. Diet, diseased state, and food intake influence the pH of the gastrointestinal fluid. The change in pH along the gastrointestinal tract has been used as a means for targeting drug to the colon. The pH of various sites is illustrated in Fig. 2.

(C) Transit time to colon [20]

Gastric emptying of dosage form is highly variable and depends primarily on whether subject is fed or fasted and on the properties of the dosage form such as size and density. Under normal conditions transit time to colon is between 5 to 7 h. But this transit time varies with fed and fasted state of GIT. Under fasted state transit time is between 3 to 5 h and in fed state it is between 6 to 10 h. The movement of materials through the colon is slow and tends to be highly variable and influenced by a number of factors other than diet like mobility, stress, disease state and presence of other drugs.

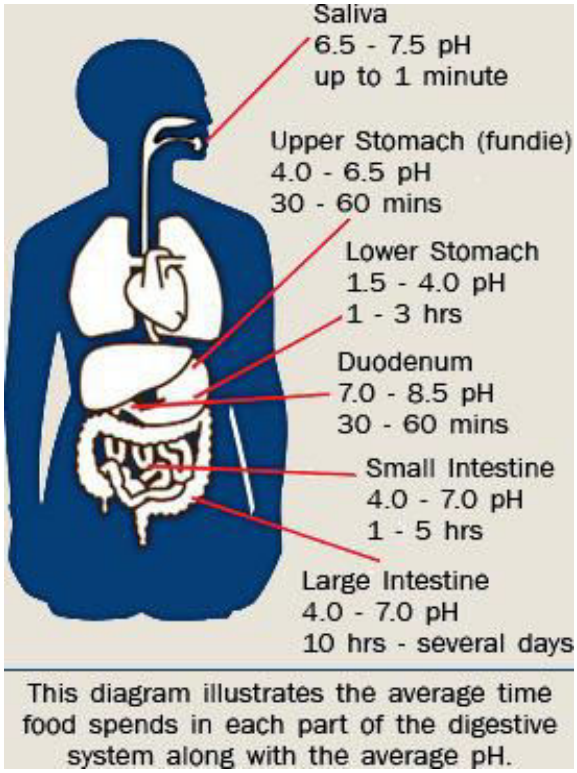

Figure: 2 pH and transit time in GIT

(D) Colonic microflora [20]

Intestinal enzymes are used to trigger drug release in various parts of the GIT. Usually, theses enzymes are derived from gut Microflora residing in high number in the colon. These facts and the bag shaped nature of the cecum make this site the favourite region for microbial settlement. Colon consists of a more than 500 different types of enzyme liberating symbiotic anaerobes. Enzymes derived from microbes are used to release drug from the polymeric coating and prodrugs. There is a vast difference in microflora count of intestine and cecum. The most important anaerobic bacteria are bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptocococcus, Ruminococcus, Propionibacterium and Clostridium. During illness and antibiotic therapy there is reversible destruction of microbes.

Classification of CTDDS:

CTDDS can be classified as follows:

- pH dependent systems.
- Time dependent systems.
- Bacterial enzyme dependent system.
- Covalent linkage of a drug with a carrier
- Redox release system.
- Bioadhesive systems.
- Coating with micro particles.
- Osmotic controlled drug delivery.

pH dependent systems [21-23]

The polymers used for colon targeting, however, should be able to withstand the lower pH values of the stomach and of the proximal part of the small intestine and also be able to disintegrate at the neutral or slightly alkaline pH of the terminal ileum and preferably at the ileocecal junction. These processes distribute the drug throughout the large intestine and improve the potential of CTDDS.

Disadvantages of pH dependent systems

Lack of consistency in the dissolution of polymer at the desired site. Moreover, many factors such as the presence of short chain fatty acids, residues of bile acids, carbon dioxide or other fermentation products can reduce the colonic pH to approximately 6 which can

certainly affect the release of drug in the colon. Certain disease state does alter the pH of the colon.

Time dependent systems [20]

Strategy of time released system is to resist the acidic environment of stomach and release the drug after predetermined lag time, after which release of drug take place.

Disadvantages of time dependent systems:

Individual to individual variation arises due to health, pathologic state, concomitant medication which causes Premature / Delayed drug release.

Bacterial enzyme dependent system [20-24]

The bio-environment inside the human GIT is characterized by the presence of complex microflora especially the colon that is rich in microorganisms that are involved in the process of reduction of dietary component or other materials. Drugs that are coated with the polymers, which are showing degradability due to the influence of colonic microorganisms, have been exploited in designing drugs for colon targeting.

Actually, upon passage of the CTDDS through the GIT, it remains intact in the stomach and small intestine where very little microbially degradable activity is present that is quiet insufficient for cleavage of polymer coating. Release of the drugs from polysaccharide based formulation is supposed to take place after degradation of polysaccharide by the enzymes released from bacterial colonies present in the colonic microflora. Some of the enzymatic degradation of polysaccharides is given in table 1.

Table:1 Microbial degradation of polysaccharides

S. NO	Polysaccharide	Bacterial species that degrade polysaccharide
1	Amylose	Bacteroids, BIfidobacterium
2	Chitosan	BIfidobacterium
3	Cyclodextran	Bacteroids
4	Guar gum	Bacteroids, Ruminococcus
5	Dextran	Bacteroids
6	Pectin	Bacteroids, BIfidobacterium, Eubacterium

Covalent linkage of the drug with a carrier [20]

It involves the formation of covalent linkage between drug and carrier in such a manner that upon oral administration the moiety remains intact in the stomach and small intestine. This approach chiefly involves the formation of prodrug, The problem of stability of certain drugs from the adverse environment of the upper GIT can be eliminated by prodrug formation. Site specific drug delivery through site specific prodrug activation may be accomplished by the utilization of some specific property at the target site.

Redox sensitive polymers

Under anaerobic conditions, bacterial azo reduction by enzymatically generated reduced flavins where the initial substrate thought to be involved in cellular electron transport requires the presence of NADPH as its electron source. As NADPH is oxidized, the electron mediator acts as an electron shuttle from the NADPH dependent flavoprotein to the azo compound. Reduction of the azo bond to the hydroazo intermediate requires a low electron density within the azo region, and thussubstitution of electron-withdrawing groups will favor this reaction. Redox potential is an expression of the total metabolic and bacterial activity in the colon and it is believed to be insensitive to dietary changes.

The mean redox potential of

- Proximal small bowl is - 67 90 mv,
- Distal small bowl is -196 97 mv
- Colon is -145 72 mv.

Microflora-induced changes in the redox potential can also be used as a highly selective mechanism for targeting to the colon.

Bioadhesive systems

Oral administration of some drugs requires high local concentration in the large intestine for optimum therapeutic effects. Dissolution of dosage form and simultaneous absorption from upper GIT lead to low intra colonic drug concentration as well as absorption of drugs result in the generation of side effects.

Bioadhesion is a process whereby drug remains in contact with a particular organ for a longer period of time. It may be used for improved absorption of poorly absorbable drugs.

Polymers: polycarbophils, polyurethanes and poloxamers.[25]

Coating with microparticles [26]

It consists of small silica particles (5-10 μm in diameter) covalently linked to a drug.

Osmotic controlled drug delivery (OROS-CT)

The OROS-CT (Alzacorporation) can be used to target the drug locally to the colon for the treatment of disease or to achieve systemic absorption that is otherwise unattainable. The OROS-CT system can be single osmotic unit or may incorporate as many as 5-6 push-pull units each 4mm in diameter, encapsulated within a hard gelatin capsule. Each bilayer push pull unit contains an osmotic push layer and a drug layer, both surrounded by a semipermeable membrane. An orifice is drilled through the membrane next to the drug layer. Immediately after the OROS-CT is swallowed, the gelatin capsule containing the push-pull units dissolves. Because of its drug-impermeable enteric coating, each push-pull unit is prevented from absorbing water in the acidic aqueous environment of the stomach and hence no drug is delivered. As the unit enter the small intestine, the coating dissolve in this higher pH environment (pH >7), water enters the unit, causing the osmotic push compartment to swell and concomitantly creates a flowable gel in the drug compartment. Swelling of the osmotic push compartment forces drug gel out of the orifice at a rate precisely controlled by the rate of water transport through the semipermeable membrane. For treating ulcerative colitis, each push pull unit is designed with a 3-4 h post gastric delay to prevent drug delivery in the small intestine.

Drug candidates suitable for colonic delivery

Drug delivery selectively to the colon through oral route is becoming increasingly popular for the treatment of large intestinal diseases and for systemic absorption of peptides and protein drugs. The best Candidates for CTDDS are drugs which show poor absorption from the stomach or intestine including peptides. The drugs used in the treatment of IBD, ulcerative colitis, diarrhea, and colon cancer are ideal candidates for local colon delivery. Drug Carrier is another factor which influences CTDDS. The selection of carrier for particular drugs depends on the physiochemical nature of the drug as well as the disease for which the system is to be used. Factors such as chemical nature, stability and partition coefficient of the drug and type of absorption enhancer chosen influence the carrier selection. The choice of drug carrier depends on the functional groups of the drug molecule.[27]

The carriers, which contain additives like polymers (may be used as matrices and hydro gels or coating agents) may influence the release properties and efficacy of the systems.

Because of small extent of paracellular transport, the colon is a more selective site for drug absorption than the small intestine. Drug shown to be well absorbed include glibenclamide, diclofenac, theophylline, ibuprofen, metoprolol, 5- flurouracil and oxprenolol. As dosage forms remain longer in the large intestine than in the small intestine, CTDDS could be used to prolong drug release. [28]

Types of formulation for colon targeted drug delivery systems [29]

Colon targeted drug delivery system have been classified into two types:

a) Single unit CTDDS: It may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon.

b) Multiparticulate CTDDS: In comparison to single unit systems, these systems offer potential benefits, like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying. Multiparticulate approaches tried for colonic delivery includes formulations in the form of pellets, granules, microparticles and nanoparticles. Multiparticulate formulations enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time, as compared to single unit systems. Because of their smaller particle size as compared to single unit dosage forms these systems tend to be more uniformly dispersed in the GIT and also ensure more uniform drug absorption.

COLORECTAL CANCER

Cancer begins when cells in a part of the body start to grow out of control. There are many kinds of cancer, but they all start because of out-of-control growth of abnormal cells. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells. In most cases the cancer cells form a tumor. Cancer cells can also invade (grow into) other tissues, something that normal cells cannot do. Growing out of control and invading other tissues are what makes a cell a cancer cell. Cells

become cancer cells because of damage to DNA. DNA is in every cell and directs all its actions. In a normal cell, when DNA is damaged the cell either repairs the damage or the cell dies. In cancer cells, the damaged DNA is not repaired, but the cell doesn't die like it should. Instead, this cell goes on making new cells that the body does not need. These new cells will all have the same damaged DNA as the first abnormal cell does.

Colorectal cancer is a term used for cancer that starts in the colon or the rectum. These cancers can also be referred to separately as colon cancer or rectal cancer, depending on where they start. Colon cancer and rectal cancer have many features in common.

Types of cancer in the colon and rectum [30]

a) Adenocarcinomas: More than 95% of colorectal cancers are a type of cancer known as adenocarcinomas. These cancers start in cells that form glands that make mucus to lubricate the inside of the colon and rectum. When doctors talk about colorectal cancer, this is almost always what they are referring to. Other, less common types of tumors may also start in the colon and rectum. These include:

b) Carcinoid tumors: These tumors start from specialized hormone-producing cells in the intestine. They are discussed in our document Gastrointestinal Carcinoid Tumors.

c) Gastrointestinal stromal tumors (GISTs): These tumors start from specialized cells in the wall of the colon called the interstitial cells of Cajal. Some are benign (non-cancerous); others are malignant (cancerous). These tumors can be found anywhere in the digestive tract, but they are unusual in the colon. They are discussed in our document Gastrointestinal Stromal Tumors (GIST).

d) Lymphomas: These are cancers of immune system cells that typically start in lymph nodes, but they may also start in the colon, rectum, or other organs. Information on lymphomas of the digestive system is included in our document Non-Hodgkin Lymphoma.

e) Sarcomas: These tumors can start in blood vessels as well as in muscle and connective tissue in the wall of the colon and rectum. Sarcomas of the colon or rectum are rare. They are discussed in our document Sarcoma - Adult Soft Tissue Cancer.

Risk factors for colorectal cancer

a) Lifestyle-related factors [30, 31]

Several lifestyle-related factors have been linked to colorectal cancer. In fact, the links between diet, weight, and exercise and colorectal cancer risk are some of the strongest for any type of cancer.

b) Personal history of inflammatory bowel disease [32]

Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease, is a condition in which the colon is inflamed over a long period of time. People who have had IBD for many years often develop dysplasia. Dysplasia is a term used to describe cells in the lining of the colon or rectum that look abnormal (but not like true cancer cells) when seen with a microscope. These cells can change into cancer over time.

c) Family history of colorectal cancer or adenomatous polyps [32]

Most colorectal cancers occur in people without a family history of colorectal cancer. Still, as many as 1 in 5 people who develop colorectal cancer have other family members who have been affected by this disease. People with a history of colorectal cancer in one or more first-degree relatives (parents, siblings, or children) are at increased risk. The risk is about doubled in those with only one affected first-degree relative. It is even higher if that relative was diagnosed with cancer when they were younger than 45, or if more than one first-degree relative is affected. Reasons for the increased risk are not clear in all cases. Cancers can "run in the family" because of inherited genes, shared environmental factors, or some combination of these.

d) Inherited syndromes [32]

About 5% to 10% of people who develop colorectal cancer have inherited gene defects (mutations) that can cause family cancer syndromes and lead to them getting the disease. These syndromes often lead to cancer that occurs at a younger age than is usual. They are also linked to other cancers besides colorectal cancer. Some of these syndromes are also linked to polyps. Identifying families with these inherited syndromes is important because it lets doctors recommend specific steps, such as screening and other preventive measures when the person is younger. The most common inherited syndromes linked with colorectal cancers are familial adenomatous polyposis (FAP) and hereditary non-polyposis

colorectal cancer (HNPCC), but other rarer syndromes can also increase colorectal cancer risk.

e) Familial adenomatous polyposis (FAP) [32]

FAP is caused by changes (mutations) in the APC gene that a person inherits from his or her parents. About 1% of all colorectal cancers are due to FAP. The most common type of FAP causes people to develop hundreds or thousands of polyps in their colon and rectum, usually in their teens or early adulthood. Cancer usually develops in 1 or more of these polyps as early as age 20. By age 40, almost all people with this disorder will have developed colon cancer if the colon isn't removed first to prevent it. Polyps that can turn into cancer can also develop in the stomach and small intestine.

f) Hereditary non-polyposis colon cancer (HNPCC) [33]

HNPCC, also known as Lynch syndrome, accounts for about 2% to 4% of all colorectal cancers. In most cases, this disorder is caused by an inherited defect in either the gene MLH1 or the gene MSH2, but other genes can also cause HNPCC. The genes involved normally help repair DNA damage. The cancers in this syndrome also develop when people are relatively young, although not as young as in FAP. People with HNPCC may also have polyps, but they only have a few, not hundreds as in FAP. The lifetime risk of colorectal cancer in people with this condition may be as high as 80%.

g) Turcotsyndrome [32]

This is a rare inherited condition in which people are at increased risk of adenomatous polyps and colorectal cancer, as well as brain tumors. There are actually 2 types of Turcot syndrome:

- One can be caused by gene changes similar to those seen in FAP, in which cases the brain tumors are medulloblastomas.
- The other can also be caused by gene changes similar to those seen in HNPCC, in which cases the brain tumors are glioblastomas.

h) Racial and ethnic background [32]

African Americans have the highest colorectal cancer incidence and mortality rates of all racial groups in the United States. The reasons for this are not yet understood. Jews of Eastern European descent (Ashkenazi Jews) have one of the highest colorectal cancer risks of

any ethnic group in the world. Several gene mutations leading to an increased risk of colorectal cancer have been found in this group. The most common of these DNA changes, called the I1307K APC mutation, is present in about 6% of American Jews.

i) Type 2 diabetes [34]

People with type 2 (usually non-insulin dependent) diabetes have an increased risk of developing colorectal cancer. Both type 2 diabetes and colorectal cancer share some of the same risk factors (such as excess weight). But even after taking these factors into account, people with type 2 diabetes still have an increased risk. They also tend to have a less favorable prognosis (outlook) after diagnosis.

EPIDEMIOLOGY

Colorectal cancers remain the third most common cancer and the third most common cause of cancer-related mortality in the US. The American Cancer Society estimates that 93,090 individuals will be diagnosed with colon cancer in the United States in 2015. Combined estimates for colon and rectal cancer are for 49,700 deaths in 2015. Worldwide, colorectal cancer is the second most common in cancer in women (614,000 cases, 9.2% of all cancers) and the third most common in men (746,000 cases, 10.0% of the total). Geographically, the incidence varies as much as 10-fold. The highest estimated rates are in Australia/New Zealand (per 100,000 population, 44.8 in men and 32.2 in women), and the lowest in Western Africa (per 100,000 population, 4.5 in men and 3.8 in women). Mortality is lower (694,000 deaths, 8.5% of the total) with more deaths (52%) in the less developed regions of the world, reflecting a poorer survival in these regions. There is less variability in mortality rates worldwide (six-fold in men, four-fold in women), with the highest estimated mortality rates in both sexes in Central and Eastern Europe (20.3 per 100,000 for men, 11.7 per 100,000 for women), and the lowest in Western Africa (3.5 and 3.0, respectively)

Signs and symptoms of colorectal cancer [35]

Colorectal cancer may cause one or more of the symptoms below. If you have any of the following you should see your doctor:

- A change in bowel habits, such as diarrhea, constipation, or narrowing of the stool, that lasts for more than a few days
- A feeling that you need to have a bowel movement that is not relieved by doing so

- Rectal bleeding
- Blood in the stool which may make it look dark
- Cramping or abdominal (belly) pain
- Weakness and fatigue
- Unintended weight loss

Colorectal cancers can bleed. While sometimes the blood can be seen or cause the stool to become darker, often the stool looks normal. The blood loss can build up over time, though, and lead to low red blood cell counts (anemia). Sometimes the first sign of colorectal cancer is a blood test showing a low red blood cell count. Most of these problems are more often caused by conditions other than colorectal cancer, such as infection, hemorrhoids, irritable bowel syndrome, or inflammatory bowel disease. Still, if you have any of these problems, it's important to see your doctor right away so the cause can be found and treated, if needed.

DIAGNOSIS [36]

a) Medical history and physical exam.
b) Blood tests
    a. Complete blood count (CBC)
    b. Liver enzymes
    c. Serum chemistries
    d. Renal function tests
    e. Tumor markers

Colorectal cancer cells sometimes make substances called tumor markers that can be found in the bloodstream. The most common tumor markers for colorectal cancer are carcino embryonic antigen (CEA) and CA 19-9.

In case of positive results of the physical exam or blood tests, colorectal cancer might be present, and can be confirmed by recommending more tests. This most often is colonoscopy, but sometimes other tests may be done first.

- Colonoscopy
- Chest x-ray
- Biopsy
- Gene test

- Microsatellite instability (MSI) testing
- Imaging tests
- Magnetic resonance imaging (MRI) scan
- Computed tomography (CT or CAT) scan: General

a) CT with portography: Checks veins and blood vessels

b) CT-guided needle biopsy: Suspected area of cancer lies deep within the body.

- Ultrasound

a) Endorectal ultrasound

b) Intraoperative ultrasound

- Positron emission tomography (PET) scan
- Angiography

TREATMENT

After the cancer is found and staged, your cancer care team will discuss your treatment options with you. The main types of treatment that can be used for colon and rectal cancer are:

- Surgery for colon and rectal cancer Surgery is the only curative modality for localized colon cancer (stage I-III). Surgical resection potentially provides the only curative option for patients with limited metastatic disease in liver and/or lung (stage IV disease), but the proper use of elective colon resections in non-obstructed patients with stage IV disease is a source of continuing debate.

- Radiation therapy although radiation therapy remains a standard modality for patients with rectal cancer, it has only a limited role in colon cancer. Radiation therapy is not used in the adjuvant setting, and in metastatic settings it is used only for palliative therapy in selected metastatic sites such as bone or brain metastases. Newer, more selective ways of administering radiation therapy, such as stereotactic radiotherapy and tomotherapy, are currently being investigated. In the future, these techniques may extend the indications for radiotherapy in the management of colon cancer.

- Chemotherapy [37-39] 5-Fluorouracil remains the backbone of chemotherapy regimens for colon cancer, both in the adjuvant and metastatic setting. In addition to 5-fluorouracil, oral fluoropyrimidines such as capecitabine (Xeloda) and tegafur are increasingly used as monotherapy or in combination with oxaliplatin (Eloxatin) levamisole and irinotecan (Camptosar) The standard chemotherapy for patients with stage III and some patients with stage II colon cancer for the last two decades consisted of 5-fluorouracil in combination with adjuncts such as levamisole and leucovorin. This approach has been tested in several large randomized trials and has been shown to reduce individual 5-year risk of cancer recurrence and death by about 30%.classification of drugs used in treatment of colorectal cancer.

Commonly used combination regimens for adjuvant therapy include the following [40, 41]

a) 5-Fluorouracil + leucovorin (weekly schedule, low-dose leucovorin)
   - 5-Fluorouracil: 500 mg/m2 intravenous (IV) weekly for 6 weeks
   - Leucovorin: 20 mg/m2 IV weekly for 6 weeks, administered before 5-fluorouracil
   - Repeat cycle every 8 weeks for a total of 24 weeks

b) 5-Fluorouracil + levamisole (weekly schedule, low-dose levamisole)
   - 5-Fluorouracil : 450 mg/m2 intravenous (IV) weekly once for 6 weeks
   - Levamisolehydrochloride : 50 mg T.I.D. daily for 6 weeks,
   - Repeat cycle every 8 weeks for a total of 24 weeks

c) Oxaliplatin + 5-fluorouracil + leucovorin (FOLFOX4)
   - Oxaliplatin: 85 mg/m2 IV on day 1
   - 5-Fluorouracil: 400 mg/m2 IV bolus, followed by 600 mg/m2 IV continuous infusion for 22 hours on days 1 and 2
   - Leucovorin: 200 mg/m2 IV on days 1 and 2 as a 2-hour infusion before 5-fluorouracil Repeat cycle every 2 weeks for a total of 12 cycles Commonly used combination regimens for metastatic disease include the following

   a) Irinotecan + 5-fluorouracil + leucovorin (FOLFIRI regimen)
      - Irinotecan: 180 mg/m2 IV on day 1
      - 5-Fluorouracil: 400 mg/m2 IV bolus on day 1, followed by 2400 mg/m2 IV continuous infusion for 46 hours
      - Leucovorin: 400 mg/m2 IV on day 1 as a 2-hour infusion, prior to 5-fluorouracil
      - Repeat cycle every 2 weeks

b) Oxaliplatin + 5-fluorouracil + leucovorin (FOLFOX6)

c) Oxaliplatin: 85-100 mg/m2 IV on day 1

- 5-Fluorouracil: 400 mg/m2 IV bolus on day 1, followed by 2400 mg/m2 IV continuous infusion for 46 hours
- Leucovorin: 400 mg/m2 IV on day 1 as a 2-hour infusion, before 5-fluorouracil
- Repeat cycle every 2 weeks c) Capecitabine + oxaliplatin (XELOX)
- Capecitabine: 850-1000 mg/m2 PO bid on days 1-14
- Oxaliplatin: 100-130 mg/m2 IV on day 1
- Repeat cycle every 21 days d) FOLFOX4 + bevacizumab
- Oxaliplatin: 85 mg/m2 IV on day 1
- 5-Fluorouracil: 400 mg/m2 IV bolus, followed by 600 mg/m2 IV continuous infusion on days 1 and 2
- Leucovorin: 200 mg/m2 IV on days 1 and 2 as a 2-hour infusion before 5-fluorouracil Bevacizumab: 10 mg/kg IV every 2 weeks
- Repeat cycle every 2 weeks

LITRATURE REVIEW

1. **AnuranjitaKundu et al., (2012)** [42], This study reveals that oral controlled release of Norfloxacin can be successfully achieved by ionotropic gelation technique using sodium alginate as polymer. Prepared microbeads shown higher drug entrapment efficiency and prolonged release characteristics. Norfloxacin release from micro beads was influenced by different alginate concentrations. Among the different formulations of micro beads, A3 was estimated as best formulation because this formulation drug release was observed that drug was released in controlled manner.

2. **M. S. Shetage et al., (2015)** [43]**,** The present study is an attempt to minimize the dosing frequency and to target the Metoprolol succinate to the colon. Drug loaded pellets are coated with pH independent Eudragit RS100 and further coated with pH dependent Eudragit S100 in R and D pan coater. 3^2 full factorial design is applied to study the effect of extent of Eudragit S100 coating %w/w (X_1) and extent of Eudragit RS100 coating %w/w (X_2) as independent variables on the dependent variables (responses) are $Y_1=Q_5$ (% released after lag time of 5h) and $Y_2=Q_{90}$ (90% of drug release within 12h). The formulation were further characterized by in vitro dissolution study, drug release kinetics and micromeritic properties. 3^2 factorial designs reveals that coating level of both the coats play a significant role in drug release property of which coating level of Eudragit RS 100 was more significant after the tablet reaches colon. Design expert software gives D5 as optimized batch having 20% w/w Eudragit RS 100 and 30% w/w with S100 as the drug release was below 20% in SIF so that it can be efficiently colon targeted, and the release is sustained up to 12 hr which is desirable for twice daily dosing of metoprolol.

3. **Anuradha Mishra et al., (2008)** [44]**,** performed the Modification of Okra mucilage with acrylamide: Synthesis, characterization and swelling behavior. In the present communication, the synthesis and characterization of Okra mucilage, a food grade and water-soluble polysaccharide, based-materials are described. Okra mucilage has been modified by grafting acrylamide (AAm) for developing the new green polymeric materials of specialty applications. Grafting has been done under N_2atmosphere using redox initiator and hydrogels were prepared by using *N*,*N*-methylenebisacrylamide (NN-MBAAm) as cross linker. The effect of monomer concentration, initiator concentration, reaction time and temperature in terms of grafting efficiency (%GE), percent grafting (PG) and percent gel (%G) has been investigated. The grafted polymers and hydrogels

were characterized by SEM, XRD and FTIR techniques to study various structural aspects. The swelling behavior of the cross-linked polymeric material has also been studied as a function of time, temperature and pH. The application area of these polymers is varied from biomaterials to the wastewater treatment.

4. **S.K. Bajpai et al., (2004)** [45], Investigated the swelling/degradation behaviour of alginate beads cross-linked with Ca^{2+} and Ba^{2+} ions. Spherical beads have been prepared by ionotropic gelation of sodium alginate in the presence of $CaCl_2$ and $BaCl_2$ solutions and their swelling behavior has been studied. The barium ion-cross-linked beads exhibit almost minimum swelling of 40% in PBS at pH 7.4 but possess greater stability while calcium alginate beads exhibit nearly 160% of water uptake and subsequently dissolve. The beads appear to swell through ion-exchange process which was confirmed by monitoring the Ca^{2+} release from the calcium alginate beads. The release was found to be diffusion controlled. On treatment with 0.1 M HCl, the calcium alginate beads demonstrated a decrease in water uptake in PBS at pH 7.4 with faster degradation while for acid treated barium alginate beads, the water uptake was found to increase on treatment with HCl. When the two beads samples were put in media of continuous varying pH (to mimic the passage of beads from mouth to colon), barium alginate beads possessed greater stability, thus showing potential to be used for colon targeted oral delivery.

5. **UzmaFarooq et al., (2013)** [46]**,** This study deals with extraction and characterization of okra (Abelmoschus esculentus) mucilage as pharmaceutical excipients. Using water based extraction method, the yield of mucilage was found to be 11.44%. Characterization of the extracted mucilage was done by various parameters such as micromeritic studies, flow behaviour, organoleptic properties, surface tension, and viscosity, loss on drying, ash value and swelling index. The result showed that extracted okra mucilage exhibited good flow properties (Angle of repose 27.29°), the surface tension of 0.25% w/v solutions of mucilage was found to be 0.0405 joule/m2, total ash was 7.53% w/w, loss on drying was 9.917% and pH was found to be 7.5. Extracted mucilage was soluble in warm water while insoluble in organic solvents. This showed that this can be safely used in dosage form without causing any adverse effect.

6. **MohdSuffianYusoff et al., (2014)** [47]**,** have prepared and characterized alginate beads by drop weight. The preparation and characterization of macro alginate beads are always associated with appropriate techniques involving precise measurement of shape, size, volume and density of the products. Depending on the type of application, encapsulation of macro alginate beads can be accomplished by various techniques including chemical, ionotropic, physical and mechanical methods. This work describes a method for preparing macro alginate beads through drop weight. The macro beads (2.85–3.85 mm) were prepared via different concentrations of alginate (0.5, 1.0, 1.5 and 2.0 g/L), dripping tip size (0.04–0.14 cm) and immersion into a predetermined concentration of calcium chloride ($CaCl_2$) bath. A custom made dripping vessel fabricated from acrylic plastic, connected to an adjustable dripping clamp was used to simulate the dripping process of the molten alginate at different tip sizes. It was observed that at different dripping tips, the correction factor for the alginate slurry was found in the range of 0.73–0.83. Meanwhile, the lost factor, *KLF* was observed at 0.93–2.3 and the shrinkage factors were limited to 2.00% from the overall distributed data. It was concluded that liquid properties had no effect on the liquid lost factor. The bead size prediction for different concentrations of alginate solution was compared to the experimental data. Subsequently, it was concluded that increasing the tip size caused the bead size to deviate almost 20% when compared to the experimental and predicted values, respectively.

7. **Ameena K et al., (2010)** [48]**,** Isolated the mucilages from Hibiscus rosasinensis linn. and Okra (Abelmoschus esculentus linn.) and studied of the binding effects of the mucilage. To isolate and evaluate comparatively the binding efficacy of the mucilages obtained from the plants of Hibiscus rosasinensis and Okra (Abelmoschus esculentus). Methods: Extraction of mucilages from the leaves of Hibiscus and pods of Okra (Ladies finger) was carried out by a cold maceration process. The extracted mucilages were subjected to various physicochemical properties for its suitability as an excipient in the formulation of tablet dosage form. Different concentrations (10, 8, 5, 2 and 1% w/v) of binder solutions of Hibiscus and Okra were used for the formulation of tablets and the formulated tablets were evaluated by studying the standard parameters like diameter, thickness, weight variation, hardness, friability, disintegration and in vitro dissolution. Stability studies of the formulated tablets were conducted for four weeks. Results: The formulated tablets prepared using the mucilages of both Hibiscus and Okra had good appearance. The in vitro drug release profile of the tablets prepared using Okra mucilage had an optimum of

90% at a mucilage concentration of 1% w/v concentration mucilage itself within 4 h. Conclusions: According to the observations, the lower concentration levels of Okra can be used as an alternative binder to starch. The higher concentration levels of Okra mucilage show a slow and sustained release, and can be considered as an alternative natural excipient in the modified drug delivery systems. At the same time, the above natural excipient of Hibiscus mucilage could be used as a platform for prolonged release if its binder concentrations are increased

8. **Anbarasan B et al., (2015)** [49]**,** development of formulation and in-vitro evaluation of capecitabine loaded Fe_3O_4 nanoparticles modified with plga-peg polymer for colon cancer treatment. 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,PEGand loaded with the drug capecitabine for the targeting of colon cancer which will be distributed in the large intestine by applying, the external magneticfield. It gets localized in the area of colon cancer cells. After the applied external magnetic field, the ironoxides (Fe_3O_4 nanoparticles) get heated to 37ºC - 40ºCand the tumour cell gets destroyed. The Fe_3O_4nanoparticles were also called as super para magnetic Iron oxide nanoparticles. 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.

9. **M. S. Khan et al., (2010)** [50]**,** Developed and Evaluated p^H -Dependent Micro Beads for Colon Targeting. The purpose of this research was to develop and evaluate multiparticulate of alginate and chitosan hydrogel beads exploiting pH sensitive property for colon-targeted delivery of theophylline. Alginate and chitosan beads were prepared by ionotropic gelation method followed by enteric coating with Eudragit S100. All formulations were evaluated for particle size, encapsulation efficiency, swellability and in vitro drug release. In vitro dissolution studies performed following pH progression method demonstrated that the drug release from coated beads depends on coat weights applied and pH of dissolution media. Mechanism of drug release was found to be swelling and erosion-dependent. The studies showed that formulated alginate and chitosan beads can be used effectively for the delivery of drug to colon and a coat weight of 20% weight gain was sufficient to impart an excellent gastro resistant property to the beads for effective release of drug at higher pH values.

10. **Bagyalakshmi et al., (2011)** [51]**,** formulated physical characterization and *in-vitro* release studies of prednisolone alginate beads for colon targeting by ionotropic gelation. This article shall give an overview on drug delivery systems for new therapeutic strategies in the treatment of inflammatory bowel disease. Conventional drug delivery systems are tightly adapted from developments of colonic delivery by oral administration triggered by release mechanism owing to the physiological environment that these systems encounter in the colonic region. The newer developments in this context aim for an increased selectivity of drug delivery by targeting mechanisms which have a closer relation to patho physiological particularities of the disease. The objective of the present study was to microencapsulate the anti-inflammatory drug (prednisolone) to provide controlled release and colon targeting. Alginate beads of prednisolone were formulated by ionotropic gelation and further coated with Eudragit S-100 and the variables studied includes concentration of sodium alginate, different cross linking agents were evaluated with respect to particle size, surface characteristics entrapment efficiency and in vitro release behavior. IR spectroscopic study confirmed the absence of any drug interaction. DSC analysis revealed that the drug was uniformly dispersed in the alginate beads. The mean particle size increases with increasing the polymer concentration. The shape of alginate beads has acceptable sphericity and surfaces were rough which were confirmed by SEM photograph. The entrapment efficiency in different formulation varied from 69% to 81%. The in vitro release profiles were also altered significantly by changing various parameters. The kinetic modeling of the release data indicates that prednisolone released from alginate beads followed by Korsemeyer's model. The above observations suggest that prednisolone can be developed as colon targeting drug delivery system with sodium alginate 2.5% using Calcium chloride as cross linking agent and coated with Eudragit S-100.

11. **Md. Hassan Kawsar et al., (2011)** [52]**,** Developed and Evaluated of Diclofenac Sodium Loaded Alginate Cross-Linking Beads. Sustained-release polymeric beads containing Diclofenac sodium fabricated with sodium alginate were prepared by the ionotropic gelation method. Drugs were blended with sodium alginate in 1:1, 1:2, 1:2.5, 1:3, 1:3.5 and 2:2 ratios. Here, calcium chloride and aluminium sulphate was used as a cross-linking agent. Beads of Diclofenac sodium were prepared with different concentrations of drug, polymers and electrolytes. Prepared beads were evaluated for their drug entrapment

efficiency, loss on drying, swelling index and release behavior. The entrapment efficiency of drug in beads depended on the amount of drug and polymer ratio as well as electrolyte concentration. The percent entrapment was highest when beads were prepared with 5 % electrolyte solution. In case of calcium chloride solution with highest amount of polymer i.e.3.5 gram the entrapment efficiency was 75.12 %. But, in aluminium sulphate solution the entrapment efficiency was highest (99.06 %) when polymer amount was 2 gram. In most cases, the swelling study revealed that, up to third hour the formulations swelled high, but swelling started to decrease after fourth hour. In case of loss on drying of beads after formation showed that, the rate of solvent loss until three hours eventually continued to increasing but then decreased. Invitro dissolution data showed that, with increasing drug, polymer and electrolyte amount the Diclofenac release percentage also decreased. Among the sixteen formulations, nine of them followed Higuchi release kinetics. Thus, by modifying the polymer amount and the selection of cross linking agent plays a vital role in efficiency and sustained-release characteristics.

12. **EhsanTaghizadehDavoudi et al., (2013)** [53]**,** Prepared and Characterized Gastric Floating Dosage Form of Capecitabine Gastrointestinal disturbances, such as nausea and vomiting, are considered amongst the main adverse effects associated with oral anticancer drugs due to their fast release in the gastrointestinal tract (GIT). Sustained release formulations with proper release profiles can overcome some side effects of conventional formulations. The current study was designed to prepare sustained release tablets of Capecitabine, which is approved by the Food and Drug Administration (FDA) for the treatment of advanced breast cancer, using hydroxypropyl methylcellulose (HPMC), carbomer 934P, sodium alginate, and sodium bicarbonate. Tablets were prepared using the wet granulation method and characterized such that floating lag time, total floating time, hardness, friability, drug content, weight uniformity, and in vitro drug release were investigated. The sustained release tablets showed good hardness and passed the friability test. The tablets floating lag time was determined to be 30–200 seconds, and it floated more than 24 hours and released the drug for 24 hours. Then, the stability test was done and compared with the initial samples. In conclusion, by adjusting the right ratios of the excipients including release-retarding gel-forming polymers like HPMC K4M, Na alginate, carbomer934P, and sodium bicarbonate, sustained release Capecitabine floating tablet was formulated.

13. **Suchitra.Domala et al., (2013)** [54], Formulation And Evaluation Of Sustained Release Tablets Of Capecitabine Using Different Hydrophylic Polymers. The objective of this work was to formulate and evaluate the sustained release matrix tablets of Capecitabine - an anti-cancer drug used in the treatment of metastatic breast cancer and colorectal cancers by using various hydrophilic polymers such as Xanthan gum, Carbopol and hydroxy propyl methyl cellulose K 100 [HPMC K 100] as cost-effective, nontoxic, easily available, with suitable hydrophilic matrix systems. Matrix tablets of Capecitabine were prepared by wet granulation method. Granules were evaluated for pre compression parameters such as bulk density, tapped density were found within limits. Angle of repose showed that the blend was freely flowing and Carr's index was in 11.29 ± 0.324 to 14.53 ± 0.926 showing that the powered blend were having good compressibility. The prepared tablets were evaluated for various post compression parameters such as hardness, friability, uniformity of weight were showed good physical properties by satisfying with the limits. The uniformity of drug content was found to be 99.63 ± 0.65% to 99.08 ± 0.28% indicating that the drug content was uniform in all batches. The dissolution test was performed in the phosphate buffer media (pH 6.8) up to 24 hours. Among the different formulations prepared, formulation no. 2 with HPMC K 100 10%, Xanthan gum 10% has the % drug release 98.44% up to 24 hours was found to be satisfactory compare to other formulations. Overall, the safety and patient compliance was improved as well as the efficacy of the drug; this was achieved by reducing the frequency of drug administration and better control of drug plasma levels.

14. **S. Latha et al., (2012)** [55]**,** formulated and evaluated capecitabine nanoparticles for cancer therapy. The main objective of this study is to formulate the Capecitabine loaded nanoparticles of chitosan (CS) cross-linked with Tripolyphosphate (TPP) for anti-cancer therapy, in order to enhance bioavailability and to reduce dose frequency. Formulation of capecitabine loaded CS/TPP nanoparticles solution was prepared by dissolving chitosan (CS) in 1% (w/v) acetic acid solution under stirring at room temperature. The CS solution was diluted with deionized water to produce different concentration. CS/TPP nanoparticles were prepared according to the ionotropic gelation process. For preparation of Capecitabine loaded CS/TPP nanoparticles, the capecitabine solution with various concentrations was added slowly to CS solution and TPP solution was added drop wise to the mixture with mild stirring for 60 min. The prepared nanoparticles were characterized by FT-IR spectroscopy to confirm the cross linking reaction between CS and cross

linking agent. X-ray diffraction was performed to reveal the crystalline nature of the drug after encapsulation. Capecitabine were loaded into the nanoparticles and the average size was found to be in the range of 120-250 nm. The Polydispersity index of the nanoparticles was found to be 0.200-0.400. The nanoparticles formed were spherical in shape with high zeta potentials (higher than +20 to +32). In vitro release studies in phosphate buffer saline (pH 7.4) showed an initial burst effect and followed by a slow drug release. The drug release followed zero order kinetics and a Fickian transport mechanism. From all these results it is concluded that the six formulations are recommended for future studies like Nano dry powder preparation. Drug loading capacity was determined by UV spectrophotometer at 240nm. The formulation was optimized for their particle size, drug release and entrapment efficiency for three independent variables by Box-Bekhen design.

15. **Melina Arnold et al., (2016)** [(56)]**,** Global patterns and trends in colorectal cancer incidence and mortality. The global burden of colorectal cancer (CRC) is expected to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030. In this study, we aim to describe the recent CRC incidence and mortality patterns and trends linking the findings to the prospects of reducing the burden through cancer prevention and care.

16. **R. Ndjouenkeu et al., (2011)** [(57)]Rheology of okra (Hibiscus esculentus L.) and dika nut (Irvingiagabonensis) polysaccharides. Polysaccharide extracts were prepared from two traditional food thickeners with extensive domestic use in central and western parts of Africa: okra (Hibiscus esculentis L.) and the seed kernel from 'dika nut' (Irvingiagabonensis). Both demonstrated typical polyelectrolyte behaviour in solution, and were therefore studied under fixed ionic conditions (0.1 M NaCl), yielding intrinsic viscosities of [η] = 7.6 dl g−1 for okra and [η] = 4.4 dl g−1 for dika. Concentrated solutions gave mechanical spectra typical of entangled networks, with close Cox-Merz superposition of η(ω) and η(γ). The variation of 'zero-shear' specific viscosity with degree of space-occupancy (c[η]) was also broadly similar to the general form observed for most disordered polysaccharides, but with greater separation of c∗ and c∗∗ and steeper slope of log ηsp vs. log c above c∗ (~4.0 for okra and ~4.6 for dika, in comparison with the usual value of ~3.3). As found for normal disordered polysaccharides, the shear-thinning behaviour of dika gum could be reduced to a single 'master-curve' for all

concentrations above c∗∗, but the absolute value of the terminal slope of log (η-ηs) vs. log ≥g was unusually low (~0.58, in comparison with the normal value of ~0.76). Terminal slopes for okra gum were also unusually low, and varied systematically with polymer concentration. These departures from normal solution properties are tentatively ascribed to compact macromolecular structures, coupled, in the case of okra gum, with a strong tendency to self-association.

17. **C.Radhika et al., (2015)** [58]**,** Formulated and In-Vitro Evaluated Of Microspheres Embedded With Capecitabine. Recent drug discovery using advanced techniques such as genomics, combinatorial chemistry, high throughput screening and in silico three dimensional drug design has yielded drug candidates with low water solubility and thus an inherently low mucosal permeability which makes the development of pharmaceutical formulations difficult. To overcome these, particulate systems like micro particles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of micro particles for drug delivery research to increase therapeutic benefit, while minimizing side effects. The review embraces various aspects of micro particle formulations, characterization, effect of their characteristics and their applications in delivery of drug molecules and therapeutic genes.

18. **UbaidullaUdhumansha et al., (2007)** [59], Developed and characterized chitosan succinate microspheres for the improved oral bioavailability of insulin. The present study describes the fabrication of insulin loaded chitosan succinate microspheres to improve the efficacy of orally administered insulin. Chitosan succinate polymer was synthesized and its microspheres were prepared by emulsion phase separation technique. The microspheres were characterized by FT-IR spectroscopy, scanning electron microscopy, particle size, X-ray diffraction, and swelling index. Insulin was loaded into the microspheres by passive absorption technique. The ability of microspheres to protect insulin from gastric enzymatic degradation was investigated. Stability of insulin in the microspheres was determined by gel electrophoresis and circular dichroism (CD). In vitro release studies were performed under simulated gastric and intestinal pH conditions (pH 2.0 and pH 7.4). The pharmacokinetic parameters were monitored after oral

administration of insulin loaded chitosan succinate microspheres, chitosan succinate–insulin solution, as well as after subcutaneous injection of insulin to diabetic rats. The degree of succinate substitution in the synthesized polymer was 16%. The prepared microspheres were spherical with an average diameter of 49 mm. The insulin-loading capacity was 62%. Chitosan succinate microspheres were found to protect the degradation of insulin from gastric enzymes. The encapsulated insulin was quickly released in simulated intestinal fluid (SIF, pH 7.4), whereas a small fraction of insulin was released in simulated gastric fluid (pH 2.0). The relative pharmacological efficacy for chitosan succinate microspheres (16%) was almost fourfold higher than the efficacy of the chitosan succinate–insulin solution administration (4%). The results suggest that chitosan succinate microspheres could be used as a potential carrier for oral insulin delivery.

19. **MerveOlukman et al., (2012)** [60]**,** Release of Anticancer Drug 5-Fluorouracil from Different Ionically cross-linked Alginate Beads. In this research, the release of 5-Fluorouracil (5-FU) from different ionically cross-linked alginate (Alg) beads was investigated by using Fe^{3+}, Al^{3+}, Zn^{2+} and Ca^{2+} ions as crosslinking agent. The prepared beads were characterized by Fourier Transform Infrared Spectroscopy (FTIR) Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM). The drug release studies were carried out at three pH values 1.2, 6.8 and 7.4 respectively each for two hours. The effects of the preparation conditions as cross linker type, drug/polymer (w/w) ratio, cross linker concentration and time of exposure to cross-linked on the release of 5-FU were investigated for 6 hours at 37˚C. It was observed that 5-FU release from the beads followed the order of Fe > Zn > Al > Ca-Alg and increased with increasing drug/polymer ratio. At the end of 6 hours, the highest 5-FU release was found to be 90% (w/w) for Fe-Alg beads at the drug/polymer ratio of 1/8 (w/w), cross linker concentration of 0.05 M, exposure time of 10 minutes respectively. The swelling measurements of the beads supported the release results. Release kinetics was described by Fickian and non-Fickian approaches.

20. **Priyanka Sinha et al., (2015)** [61]**,** Zinc (Zn^{2+})-ion induced diclofenac sodium (DS)-loaded alginate-okra (Hibiscus esculentus) gum (OG) blend beads was successfully formulated through Zn^{2+}-ion induced ionic-gelation cross-linking method in a complete aqueous environment. Effects of polymer-blend ratio and cross-linker concentration on drug encapsulation efficiency (DEE) and cumulative drug release at 8 h (R8h) were

optimized by 3^2-factorialdesign. The optimized formulation of Zn^{2+}-ion induced DS-loaded alginate-OG beads demonstrated 89.27 ± 3.58% of DEE and 43.73 ± 2.83% of R8h. The bead sizes were within 1.10 ± 0.07 to 1.38 ± 0.14 mm. The bead surface morphology was analyzed by SEM. The drug–polymer interaction in the optimized bead matrix was analyzed by FTIR and P-XRD. These beads exhibited sustained in vitro drug release over a prolonged period of 8 h and followed controlled-release (zero-order) pattern with super case-II trans-port mechanism. The swelling and degradation of the optimized beads was influenced by the pH of test mediums, which might be suitable for intestinal drug delivery.

21. **G. Pavani et al., (2013)** [62]**,** Formulated And Evaluated Of Capecitabine Sustained Release Tablets. 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. Totally 9 formulations were prepared. 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. The results of in vitro drug release studies showed that formulation-2 (API and HPMC and Xantham gum) has better drug release (98.44%) for 24hrs.

22. **Divyen Shah et al., (2011)** [63]**,** designed a study to investigate the combined influence of three independent variables on two dependent variables for the preparation of nanoparticles of levamisole hydrochloride. The nanoparticles were prepared by ionotropic gelation method. The three independent variables are the chitosan/sodium tripolyphosphate ratio, pH of sodium tripolyphosphate and levamisole concentration; while particle size and percentage drug entrapment were dependent variables. The nanoparticles were prepared by using 2^3 factorial design to obtain high entrapment efficiency and small size. The polynomial equation can be used to predict the responses. The prepared nanoparticles show busted release for the first 2 h and then show sustained release. The differential scanning calorimetry graphs indicate that drug is completely entrapped in nanoparticles and transmission electron microscopy images show that nanoparticles are spherical in shape and dense solid in nature. The mathematical model

obtained by 2^3 factorial design shows good relationship between independent variables and dependent variables for prediction.

23. **HarpreetKaur et al., (2013)** [64]**,** investigated colonic delivery using shellac as coat over the hard gelatin capsule. The drug delivery system was based on the gastrointestinal transit time concept, assuming colon arrival time to be 6 h. Rapidly disintegrating capsules containing 50mg 5-FU were coated with different concentration of shellac by dipping method. In order to find the suitable formulation, various formulation factors were investigated through series of in-vitro dissolution studies in buffer solution at pH 1.2 for first 2 h, and at pH 6.8 for remaining hour. The results indicated that C4 is the most suitable formula in the approach of time dependent oral delivery system for colon targeting for achieving minimum release in the first five hours and maximum at the 24 th hour. The formulation C4 was then studied with different probiotics. Gp1 shows a release of 12.14 % in first five hours but, on 24 hours Gp1 shows a maximum release of 98.75 %. So the capsule with probiotc was found to be more effective than without probiotics.

24. **KuntalGanguly et al., (2011)** [65]**,** formulated 5-Flurouracil spherical microspheres by loading different concentrations of 5-Flurouracil (10, 20, and 30 %w/w) into polyethylene glycol cross-linked chitosan microspheres by using emulsion cross linking technique and then enteric coated it with cellulose acetate phthalate to facilitate drug targeting to the colon. The microspheres were evaluated for swelling index, encapsulation efficacy and in-vitro release characterization in simulated gastrointestinal tract conditions. The uncoated released 46-77% in acidic pH and 93-97% in alkaline pH whereas the CAP coated microspheres released 8-17% in acidic pH and 88-97% in alkaline pH. The study concluded that chitosan microspheres cross linked with PED and coated with CAP showed better colon targeting activity as they demonstrated better prolonged release for 6-12 hrs by protecting from acidic environment of stomach. Kinetic studies showed that the release of drug is by super case II transport mechanism.

25. **Ramana G et al,. (2011)** [66]**,** prepared and characterized pectin alginate microspheres loaded with 5-Flurouracil and coated with ethyl cellulose for colon targeting. The microspheres were prepared by ionotropic gelation method and coated with ethyl cellulose. The various characterization parameters evaluated were drug-excipientcompatability, drug entrapment effeciency, swellability, surface morphology,

invitro release studies and stability studies. DSC showed no interaction between the drug and excipients and hence was used in the formulation. Optimized formulation showed 69.94% drug entrapment. The in-vitro release studies were carried out in the presence and absence of pectinase enzyme and found that the drug release was 93.25% and 97.82% respectively. The stability studies showed that there was no degradation of the formulation. Hence the experimental results demonstrated that ethyl cellulose-coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

26. **Sridar et al., (2010)** [67]**,** developed and evaluated Multiparticulates of alginate and chitosan hydrogel beads exploiting pH sensitive property for colon-targeted delivery of theophylline. Alginate and chitosan beads were prepared by ionotropic gelation method followed by enteric coating with Eudragit S100. All formulations were evaluated for particle size, encapsulation efficiency, swellability and in vitro drug release. In vitro dissolution studies performed following pH progression method demonstrated that the drug release from coated beads depends on coat weights applied and pH of dissolution media. Mechanism of drug release was found to be swelling and erosion-dependent. Coated chitosan beads released more than 70% and coated alginate beads released more than 80% of drug in pH more than 7.0 while in acidic pH chitosan and alginate beads released 8% and 6 % respectively with a coat weigh of 20%. The studies showed that formulated alginate and chitosan beads can be used effectively for the delivery of drug to colon and a coat weight of 20% weight gain was sufficient to impart an excellent gastro resistant property to the beads for effective release of drug at higher pH values.

27. **Bose A et al., (2007)** [68]**,** prepared 5-flurouracil pellets coated with pectin and ethyl cellulose for the treatment of colon cancer. They studied pharmacodynamic and pharmacokinetic profiles of intracapsular coated pellets invivo in rats with reference to their to their site specific drug release outcomes. The pellets were prepared by extrusion spheronozation technique. In-vitro drug release, drug content, invivo pharmacokinetics, local colonic drug content, systemic haematology and clinical chemistry profiles of coated and uncoated pellets were examined against unprocessed drug. Invivo coated pellets reduced drug bioavailability and enhanced the drug acculmulation in the colon (179.13 µg of 5-FU/g rat colon content vs 4.66 µg of 5-FU/g rat colon content of conventional in-vivo film coated pellets at dose of 15mg/kg dose). The coated pellets

showed reduction in tumor size and number by reforming tubular epithelium with epithelium membrane and restricting expression of cancer from adenoma to adenocarcinoma. The coated pellets eliminated aberrant crypt foci which represented a pulsatile preneoplastic lesion in colon cancer. They did not implicit additional systemic toxicity.

28. **NandgudeTanajiDilip et al., (2016)** [69]**,** Formulated and Developed Colon Specific Multiparticulate System of Capecitabine. In the present study, the main objective was to develop a multiparticulate system containing chitosan microspheres for colon-specific drug delivery of capecitabine for the treatment of colorectal cancer. Materials and Methods: This study was based on the microbial degradability of chitosan microspheres. The microspheres were prepared with chitosan by emulsion cross linking method. A factorial design was applied to optimize the formulation. The effect of concentration of chitosan and drug: Polymer ratio was studied on particle size, % entrapment efficiency, and % drug release using 3^2 factorial designs. Results and Discussion: 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. Conclusion: In vitro drug release studies were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract showed a fast drug release initially demanded microencapsulation.

29. **EhsanTaghizadehDavoudi et al., (2013)** [70]**,** Preparation and Characterization of a Gastric Floating Dosage Form of Capecitabine. Gastrointestinal disturbances, such as nausea and vomiting, are considered amongst the main adverse effects associated with oral anticancer drugs due to their fast release in the gastrointestinal tract (GIT). Sustained release formulations with proper release profiles can overcome some side effects of conventional formulations. The current study was designed to prepare sustained release tablets of Capecitabine, which is approved by the Food and Drug Administration (FDA) for the treatment of advanced breast cancer, using hydroxypropyl methylcellulose (HPMC), carbomer934P, sodium alginate, and sodium bicarbonate. Tablets were prepared using the wet granulation method and characterized such that floating lag time, total floating time, hardness, friability, drug content, weight uniformity, and in vitro drug release were investigated. The sustained release tablets showed good hardness and passed the friability test. The tablets' floating lag time was determined to be 30–200 seconds, and

it floated more than 24 hours and released the drug for 24 hours. Then, the stability test was done and compared with the initial samples. In conclusion, by adjusting the right ratios of the excipients including release-retarding gel-forming polymers like HPMC K4M, Na alginate, carbomer934P, and sodium bicarbonate, sustained release Capecitabine floating tablet was formulated.

30. **PayamKhazaeli et al., (2013)** [71], Formulated Ibuprofen Beads by Ionotropic Gelation. Microencapsulation has become a common technique in the production of controlled release dosage forms. Many results have been reported, concerning the use of alginate beads as controlled release drug formulations. Alginate has a unique gel-forming property in the presence of multivalent cations, in an aqueous medium. Ibuprofen is an excellent analgesic and antipyretic, non-steroidal anti-inflammatory agent with a high therapeutic index. Formulation of ibuprofen in beads could reduce its gastric ulcerogenicity. Hence, in this study the formation of Ca-alginate ibuprofen beads, through ionotropic gelation has been investigated. For this purpose, different cross- linking agents including: Ca^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Sn^{2+} and Pb^{2+}, were used for bead preparation . Next, characterization of the beads, size distribution, and encapsulation efficiency of ibuprofen within the beads, the bead swelling and the drug release kinetic were investigated. Results showed that only Ca ion is suitable for the formation of ibuprofen beads. A good swelling profile for beads in phosphate buffer (pH=7.4) and the lack of swelling in hydrochloric acid (pH= 1.2), show the suitable nature of the beads. In addition, formulation of Na-alginate (2%) and Ca-chloride (2%) beadsresulted in an encapsulation efficacy of around 90%. The drug release studies showed a rapid and complete ibuprofen release from the beads, especially those prepared from Na-alginate (2%) and Ca-chloride (2%), in phosphate buffer medium. However, no detectable drug release was observed within the acidic medium.In conclusion, ibuprofen is capable of being n be microencapsulated as a bead formulation,with suitable properties and release profile.

AIM AND OBJECTIVE

Alginates are generally regarded as safe (GRAS) by the FDA .Sodium alginate (SA) is the sodium salt of alginic acid, which is aco-polymer of β-d-mannuronic acid (M) and ∞-l-guluronic acid(G) having 1,4-glycosidic linkage between them. It has been used in various pharmaceutical applications. It produces cured gel matrices in the presence of multivalent cations like Ca^{2+}, Cd^{2+},Ba^{2+}, Zn^{2+}, Al^{3+}, etc., due to intermolecular ionic-gelation cross-linking between COO−groups located on the SA backbone and these metal cations. Utilizing this property of SA, various alginate gel beads were prepared for the delivery of various drugs. Though these alginate beads can be prepared easily through simple and economic procedures, these beads suffer from low drug encapsulation and poor mechanical property in the intestinal pH, which lead rapid drug release. To minimize these limitations, ionically gelled alginate beads have been modified by various research groups. Currently, modified alginate beads using SA and plant-derived natural polysaccharide blends have been investigated to improve drug encapsulation, swelling, drug release, etc. Most of these modified alginate beads were prepared using plant-derived natural polysaccharide blends with SA and using calcium chloride ($CaCl_2$) as a cross-linker. Various calcium-ion induced alginate-based beads loaded with drugs were also investigated earlier. In the current communication, we aimed to develop controlled drug release Ca^{2+}-ion induced alginate-okra gum (OG) blend beads by ionic-gelation cross-linking method using calcium chloride ($CaCl_2$) as a cross-linker in an aqueous environment.

Okra (Hibiscus esculentus, family – Malvaceae) is an annual plant cultivated throughout the tropical and subtropical areas of the world. The immature okra fruit is also used in folk medicine. OG obtained from okra fruit is composed of d-galactose,l-rhamnose and l-galacturonic acid. It is chemically inert, non-irritant, biodegradable and biocompatible. It is water-soluble and produces highly viscous solution with a slimy appearance. OG is already investigated as useful excipients in the development of various pharmaceutical formulations. The highly viscous property of OG leads the usefulness of it as a drug-release retarding polymer. Capecitabine is a prodrug that is selectively tumour-activated to its cytotoxic moiety, fluorouracil, by thymidine phosphorylase. 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 triphosphate. The active moiety of capecitabine, fluorouracil, is cell cycle phase-specific (S-phase).

In order to utilize the OG for the oral drug delivery, the development of other dosage forms, which is more convenient in administration, is demanded. Bead-type of OG gel has several advantages such as the facility of drug loading, reduction of the risk of dose dumping, and facility of dose adjustment besides the convenient administration. However, the stability of OG beads is lowered due to the isolation of gelled spheres in the dilute condition of GI track. The special coating process will improve the stability of OG beads and also allow the colon-specific release of gastric mucosal irritant drug.

Specific Eudragit polymers have been developed for oral dosage forms with step-wise release of active ingredient in the digestive tract. Eudragit is insoluble below pH 5 and thus resistant to gastric fluid. By salt formation in the neutral to weakly alkaline medium of the intestinal fluid, the polymer dissolves step-wise at pH values above 6. These properties are what render Eudragit as the most popular material for enteric-coating and stabilizing agent for OG beads.

In this study, factorial design based on response surface methodology(RSM) was introduced to optimize the preparation of OG beads.RSM is a useful statistical technique for formulation and process optimization, which is accomplished by studying the mutual interactions among the variables over a range of values in a statistically valid manner. OG beads were prepared by thermo sensitive gelation of OG aqueous solution and coated with Eudragit. In vitro drug release behavior of the Eudragit coated OG beads was carried out in gastro-intestinal pH (1.2, 5.8 and 7.4). Invitro release kinetics models were applied to the study the release mechanism of the drug from beads.

PLAN OF THE WORK

1. LITERATURE SURVEY
2. PREFORMULATION STUDIES
    a. Solubility of the drug
    b. Drug and excipient compatibility
    c. Standard graph of drug
3. PREPARATION AND CHARACTERIZATION OF OKRA GUM
4. PREPARATION AND OPTIMIZATION OF BEADS BY BOX-BEHNKEN MODEL
    a. Preparation of Sodium alginate and okra gum beads
    b. Preparation of Capecitabine loaded Sodium alginate and okra gum beads
    c. Eudragit coated Capecitabine loaded Sodium alginate and okra gum beads
5. EVALUATION OF BEADS
    a. Micrometric properties
    b. Drug entrapment efficiency and Drug loading
6. SWELLING INDEX AT DIFFERENT pH
7. INVITRO RELEASE STUDIES
    a. Preparation of Capecitabine loaded Sodium alginate and okra gum beads
    b. Eudragit coated Capecitabine loaded Sodium alginate and okra gum beads
8. INVITRO RELEASE KINETICS
    a. Zero order kinetics
    b. First order kinetics
    c. Higuchi model of kinetics
    d. Hixson Crowel kinetics
    e. Korsmeyer-Peppas model for kinetics

DRUG AND EXCIPIENT PROFILE

CAPECITABINE [72, 73]

Structure:

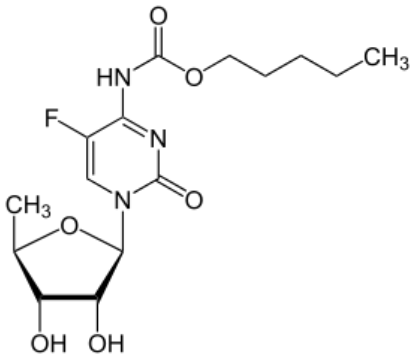

Figure: 3 STRUCTURE OF CAPECITABINE

IUPAC Name:

Pentyl [1-(3,4-dihydroxy-5-methyltetrahydrofuran-2-yl)-5-fluoro-2-oxo-1H pyrimidin-4-yl] carbamate

Molecular weight:

359.35

Molecular Formula:

$C_{15}H_{22}FN_3O_6$

Melting point:

110-121˚C

LogP:

0.4

Solubility:

It is soluble in water (2ymg/ml)

Category:

Antineoplastic

$T_{1/2}$:

Approximately 38-45 minutes

Dose:

The usual starting dose is 2,500mg/m2/day in two divided doses, 12 hours apart. One cycle includes two weeks of treatment followed by one week without treatment. Cycles can be repeated every three weeks.

BCS:

Class type III (High solubility, Low permeability)

Pharmacokinetics:

Absorption:

Readily absorbed through GI tract (approximately 70%).Time to reach peak plasma concentration for Capectabine is approximately 1.5 hours and for 5-fluorouracil is 2 hours. Food decreased peak plasma concentration is 60% and area under curve is 35% for Capecitabine and decreased peak plasma concentration (Cmax) 4.3% and area under curve 21% for 5-fluorouracil. Food delayed Tmax is 1.5 hours.

Protein binding:

Less than 60% protein binding (mainly albumin).

Metabolism:

Metabolized by thymidine phosphorylase to fluorouracil .

Elimination:

Capecitabine and its metabolites are predominantly excreted in urine. About 95.5% of administered Capecitabine dose is recovered in urine. Focal excretion is minimal (2.6%). The major metabolite excreted in urine is FBAL which represents 57% of the administered dose. About 3% of the administered dose is excreted in urine as unchanged drug.

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-methylenetetrahydrofolate, bind to thymidylate synthase (TS)to form a covalently bound ternary complex. This binding inhabits 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 inhabit cell division. Secondly, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis through the production of fraudulent RNA.

Indications and usage:

Capecitabine is a nucleoside metabolic inhibitor with anti-neoplastic activity. It is used in the treatment of adjuvant colon cancer Stage III Duke's C-used as first-line monotherapy.

Metastatic colon rectal cancer

- First line as monotherapy alone is preferred.

Metastatics breast cancer

- Asmonotherapy, if the patient has failed paclitaxel based treatment, and if anthracycline based treatment, and if anthracycline based treatment has either failed or cannot be continued the other reasons.
- Used in combination with docetaxel, after failure of anthracycline based treatment.

Adverse reactions:

Most common adverse reactions are:

Cardiovascular	: EKG changes, myocardial infarction, angina.
Dermatological	: Hand and foot syndrome.
Gastrointestinal	: Diarrhea, Nausea, stomatitis.
Heamatological	: Neutropenia, anemia and thrombocytopenia.
Hepatic	: Hyperbilirubinemia.

Drug interaction:

- **Anticolagulants** : May interact with warfarin and increase bleeding risk.
- **Phenytoin** : May inhibit cytochrome CYP2C9 enzyme, and therefore increase levels of substrates such as Phenytoin and other substrates of CYP2C9
- **Leucovorin** **:** The concomitant use of Leucovorin increased the toxicity of Capecitabine without any apparent advantage in response rate.

Pharmacodynamic:

Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity indicated for the treatment of metastatic breast cancer and colon cancer. It is an orally administered systemic prodrug 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)

POLYMER PROFILE

SODIUM ALGINATE [73]

Non-proprietary Names:

BP : Sodium Alginate
PhEur : Sodium Alginate
USP-NF : Sodium Alginate

Synonym:

Alginatosodico, algin, alginic acid, sodium salt, E401, Kelcosol, Keltone, natriialginas, Protanal, sodium polymannuronate.

Chemical Name

Sodium alginate

Functional category

Stabilizing agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity increasing agent

Structural Formula:

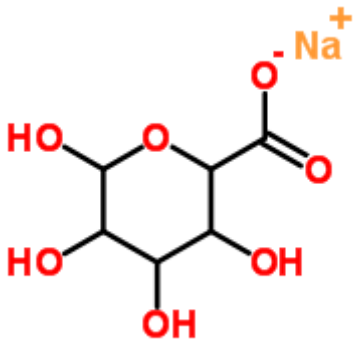

Figure: 4 Structure of Sodium Alginate

Molecular Weight:

216.121gm/mol.

Description

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder.

Solubility

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3. Slowly soluble in water, forming a viscous colloidal solution.

Viscosity

Typically, a 1% w/v aqueous solution, at 20ºC, will have a viscosity of 20– 400 mPas (20–400 cP). Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions. Above pH 10, viscosity decreases.

Incompatibilities

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenyl mercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate; salting-out occurs if more than 4% of sodium chloride is present.

Stability and storage condition

Stable, the bulk material should be stored in an airtight container in a cool, dry place.

Safety:

It is generally regarded as a nontoxic and non-irritant material, although excessive oral consumption may be harmful.

Application in pharmaceutical formulation or technology:

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrant, it has been used as a diluent in capsule formulations. Sodium alginate has also been used in the preparation of sustained-release oral formulations. In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams, and gels, and as a stabilizing agent for oil-in-water emulsions. Recently, sodium alginate has been used for the aqueous microencapsulation of drugs.

CALCIUM CHLORIDE [74]

Non-proprietary Names

- BP: Calcium Chloride Dihydrate, Calcium Chloride Hexahydrate
- JP: Calcium Chloride Hydrate
- PhEur: Calcium Chloride Dihydrate
- Calcium Chloride Hexahydrate
- USP-NF: Calcium Chloride

Synonyms

Calciichloridumdihydricum; calciichloridumhexahydricum.

Chemical Name and CAS Registry Number

- Calcium chloride anhydrous [10043-52-4]
- Calcium chloride dihydrate [10035-04-8]
- Calcium chloride hexahydrate [7774-34-7]
- Empirical 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)

Structure

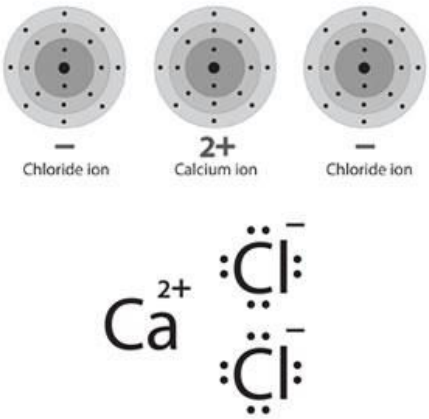

Figure: 5 Structure of calcium chloride

Functional Category

Antimicrobial preservative; therapeutic agent; water-absorbing agent.

Applications in Pharmaceutical Formulation or Technology

1. The main applications of calcium chloride as an excipients relate to its dehydrating properties and, therefore, it has been used as an antimicrobial preservative, as a desiccant, and as an astringent in eye lotions.
2. Therapeutically, calcium chloride injection 10% (as the dehydrate form) issued to treat hypocalcaemia.

Description

Calcium chloride occurs as a white or colorless crystalline powder, granules, orcrystalline mass, and is hygroscopic (deliquescent).

Pharmacopeial Specifications

Property	Limit
pH	4.5-9.2
Boiling point	>16008 ˚C (anhydrous)
Density	0.835g/cm^3 (Dihydrate)
Melting point	7728˚C (Anhydrous)
	1768˚C (Dihydrate)
	309˚C (hexahydrate)
Solidification temperature	28.5-308˚C
Solubility	Freely soluble in water and ethanol
	Insoluble in diethyl ether and diethyl ether

Stability and Storage Conditions

Calcium chloride is chemically stable; however, it should be protected from moisture.Store in airtight containers in a cool, dry place.

Incompatibilities

Calcium chloride is incompatible with soluble carbonates, phosphates, sulfates, andtartrates.

It reacts violently with bromine trifluoride, and a reaction with zinc releases explosive hydrogen gas. It has an exothermic reaction with water, and when heated to decomposition it emits toxic fumes of chlorine.

Method of Manufacture

Calcium chloride is a principal by product from the Solvay process.

Safety

Calcium chloride is used in topical, ophthalmic, and injection preparations. The pure form of calcium chloride is toxic by intravenous, intramuscular, intraperitoneal, and subcutaneous routes, and moderately toxic by ingestion, causing stomach and heart disturbances. It is a severe eye irritant and can cause dermatitis.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of the material handled. Calcium chloride is irritating to eyes, the respiratory system, and skin. Gloves, eye protection, respirator, and other protective clothing should be worn.

Regulatory Status

GRAS listed. Included in the FDA Inactive Ingredients Database (injections, ophthalmic preparations, suspensions, creams).Included in medicines licensed in the UK (eye drops; intraocular irrigation; vaccines; injection powders for reconstitution; nebulizer solution; oral suspension).

EUDRAGIT S – 100 [74]

Synonym

Eudragit; Methacrylic acid

Non-proprietary Names

NF: Methacrylic acid copolymer; Polymeric methacrylates

Chemical Name

Copolymers synthesized from dimethyl amino-ethyl methacrylate and other neutral lmethacrylic esters.

Functional category

Film former and tablet binder.

Density

12.5; 0.825g/cm^3

Structure

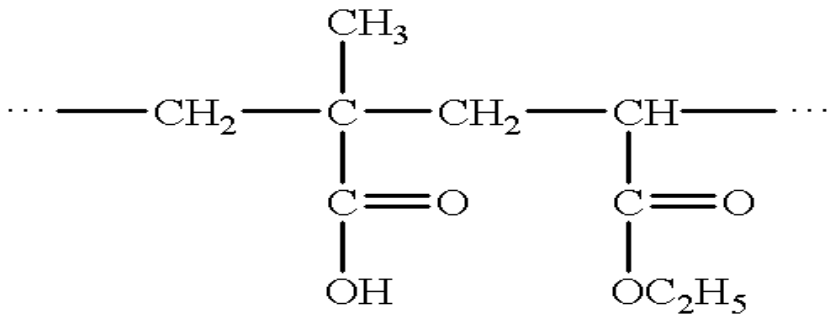

Figure: 6 Structure of Eudragit S 100

Molecular Weight

100,000 and approximately 135,000

Description

Polymethacrylates are film coatings and matrix structures based on polymericmethacrylates. They are synthetic cationic and anionic polymers of dimethyl amino ethylmethacrylates, methacrylic ratios. Type L (easily soluble in intestinal fluid) is 50% methacrylic acidand Type S(barely soluble in intestinal fluid) is 30% methacrylic acid; both areanionic polymers

of methacrylic acid and methacrylic acid esters in different ratios available as 12.5% solution in isopropanol without plasticizer (L 12.5, S 12.5); and as12.5% ready to use solution in isopropanol with 1.25% dibutylphthalate as plasticizer (L 12.5p, S 12.5p);colorless, with the characteristic odor of the solvent.

Solubility

1g of Eudragit L100 0r Eudragit S100 dissolves in 7g methanol, ethanol, in aqueous isopropyl alcohol and acetone, as well as in 1 N sodium hydroxide to give clear to slightly cloudy solutions. Eudragit L100 and Eudragit S100 are practically insoluble in ethyl acetate, methylene chloride, petroleum ether and water.

Viscosity

Eudragit S100= 29mm^2/s

Acid value

Eudragit L100=316mgKOH/gDS

Eudragit S 100=190mgKOH/gDS

Incompatibilities

Incompatibilities occur with acid and/or alkaline conditions depending upon which polymer is being used.

Stability and storage condition

Dry powder forms appear to be stable at room temperature. Dispersions are stable for about 1 yr after manufacturing and stored at room temperature in tight containers protect against moisture.

Safety

Acute toxicity studies have been performed in rats, rabbits and dogs. No toxic effects were observed. Chronic toxicity studies were performed in rats over a period of 3 months. No significant changes were found in the animal organs.

MATERIALS

List of materials and suppliers

Table 2: List of materials used

S.NO	RAW MATERIALS	MANFACTURER
1.	Capecitabine	Sigma Aldrich
2.	Eudragit S – 100	Yarrow Chem Products Mumbai, India
3.	Acetone	Rolex laboratory reagent
4.	Ethanol	Lobachemieloboratory and fine chemicals
5.	Sodium alginate	S.D Fine Chemicals, Mumbai India
6.	Calcium chloride	S.D Fine Chemicals, Mumbai India
7.	Ethanol	Lobachemieloboratory and fine chemicals

List of equipment's and manufacturer

Table 3: List of instruments used

S.NO	INSTRUMENTS	MANFACTURER
1.	Electronic balance	Wensar
2.	Digital pH meter	Elico
3.	Sonicator	Vibro cell
4.	Dissolution apparatus	Electrolab
5.	Magnetic stirrer	Remi motors
6.	Mechanical shaker	Technico lab

7.	UV spectrometer	Shimadzu, japan
8.	Fourier transit infra-red spectroscopy	Thermo scientific Nicolet ISF
9.	Scanning electron microscopy	JEOL
10.	Electronic microscope	LW electronic microscopes
11.	Hot air oven	Techno Lab

METHODS

ISOLATION OF HIBISCUS ESCULENTUS (OG) [46]

1 kg of unripe and tender Okra fruits

Washed and sliced thinly using a knife

Seeds were removed from sliced fruits

Sliced mass was soaked in distilled water

Over night

After soaking, viscous gum is filtered out using muslin cloth

To the viscous gum acetone was added to precipitate gum at a ratio of ratio of 3 parts of acetone to 1 part of the gum

Then, the precipitate was dried at 40°C. The dried film obtained was crushed to fine powder through sieve no. 120 and kept in air-tight desiccator.

Characterization of Okra gum: [46,48].

Ash Values: As discussed by authors in previous publication ash values such as total ash, acid insoluble ash and water- soluble ash were determined using equation 1, 2, 3 respectively.

$$\text{Total ash value} = \frac{weig\ htofas\ h}{weig\ htofpolymer} \times 100 \quad (1)$$

$$\text{Acid insoluble ash} = \frac{weig\ htofacidinsolubleas\ \ h}{weig\ htofdriedpowder} \times 100 \quad (2)$$

$$\text{Water soluble ash} = \frac{weig\ htofwatersolubleas\ \ h}{weig\ htofdriedpowder} \times 100 \quad (3)$$

Solubility behaviour: As already described by author's one part of dry mucilage powder was shaken with different solvents and further solubility was determined.

pH of Mucilage: The mucilage was weighed and dissolved in water separately to get a 1%w/v solution. The pH of solution was determined using digital pH meter as described by authors in previous publication.

Surface Tension: The surface tension of the selected mucilage was determined by drop count method, using astalagmometer. The surface tension of the polymer has been reported to influence binding quality of the polymer. Surface tension was calculated as per equation 4.

$$\sigma_{solution} = \sigma_{water} \frac{m(solution\ )}{m(water\ )} \quad (4)$$

Where,

$\sigma_{solution}$– Surface tension of solution

σ_{water}- Surface tension of water

m (solution)- Weight of solution

m (water)- Weight of water

Viscosity: As described by authors viscosity of okra mucilage was determined using Oswald viscometer were calculated using equation 5.

$$S = w \times \frac{ts\rho s}{tw\rho s} \quad (5)$$

Where,

s- Viscosity of solution

w- Viscosity of water

t- Time

ρ- Density

Loss on Drying: The test was carried out according to the procedure described by authors elsewhere. One gram of powder was weighed accurately in a weighing bottle and was dried in a hot air oven at 105°C and the weight was checked at intervals of 10min, until a constant weight was obtained. The percentage weight lost by the powder was calculated using equation (6).

$$\text{Loss on drying} = \frac{initialweig\ \ ht - final\ weig\ ht}{initialweig\ \ ht} \times 100 \qquad (6)$$

Bulk Density and Bulkiness: It has been described by authors that inverse of bulk density is called as bulkiness. As per previous study accurately weighed quantity of (50 g) was introduced into a graduated measuring cylinder. The cylinder was fixed on the bulk density apparatus and the volume occupied by the powder was noted. Then, the powder was subjected to tapping in a bulk density apparatus until constant volume was obtained. The final volume (Bulk volume) was noted. Bulk density, tapped density and bulkiness were calculated using equations 7-9 respectively.

$$\text{Bulk density} = \frac{weig\ htofpowderblend}{weig\ htofapparentvolume} \qquad (7)$$

$$\text{Tapped density} = \frac{weig\ htofpowderblend}{tappedvolume} \qquad (8)$$

$$\text{Bulkiness} = \frac{1}{bulkdensity} \qquad (9)$$

True Density: Among various methods available for the determination of true density, liquid displacement method is the simplest method and was used in the present study. Acetone was selected as the liquid for displacement, because, mucilage is insoluble and heavy in acetone. This method has been used by many authors.

Powder Flow Property: Flow characteristics were measured by angle of repose as previous publication of authors. Same study was repeated here. Using the readings and the formula, the angle of repose was calculated using equation 10.

$$\text{Tan } \Theta = h/r \qquad (10)$$

Where,

Θ- Angle of repose

h- Height of pile

r- Radius of pile

Powder Compressibility (Carr's Consolidation Index): This property is also known as compressibility. As described in previous publication finely powdered mucilage (5 g) was transferred into a measuring cylinder and calculations were done using bulk density apparatus.

Particle Size Analysis: The particle size was determined by microscope.

Characterization of Capecitabine

Preformulation studies:

Preformulation can be defined as an investigation of physical and chemical properties of a drug substance alone. The overall objective of preformulation studies is to generate information useful to the formulator in developing stable and bio available dosage forms.

- Organoleptic properties
- Solubility Studies

Organoleptic properties:

The organoleptic characters of the drug like colour, odor, taste and appearance play an important role in the identification of the sample and hence they should be recorded in descriptive terminology.

Solubility studies:

An excess of drug is suspended in 100ml of dissolution medium containing various concentrations of carriers in stopper flask and equilibrated by intermittent shaking for 72 hrs

maintained at 37±2^{0}c.The solution is filtered through whattman filter paper. A portion of filtrate is diluted suitably and analyzed by UV spectroscopy.

Analytical Method Development

Standard curve of drug was prepared in 0.1N HCl, 5.8 buffer and 7.4 buffer.

Procedure:

1. Preparation of stock solution: An accurately weighed 100 mg of Capecitabine was dissolved in 0.1N HCl, 5.8 phosphate buffer and 7.4 phosphate buffer separately and make up the volume up to 100 ml in a volumetric flask (Stock Solution: I, 1000 µg/ml). This was sonicated for 5 minutes.

2. From this 10 ml of solutions were pipette out and make up the volume up to 100 ml (Stock Solution: II, 100 µg / ml).

3. Then the aliquots were prepared, whose concentration ranging from 0 to 60µg/ml for 0.1N HCl, 0 to 20µg/ml for 5.8 buffer and 0 to 20µg/ml 7.4 buffer and the absorbance was measured at wavelength of 303nm by using UV Spectrophotometer (Shimadzu, Model No: 2450) against the blank.

Experimental design for statistical optimization [78]

A total of seventeen trial Capecitabine -loaded alginate-OG blend beads were proposed by three factors and two responses factorial design and prepared by ionic-gelation cross-linking method. Three factors are SA (A), OG (B) and $CaCl_2$ (C), which were varied at three different levels: low(−1), medium (0) and high (+1). Drug loading (%) and cumulative drug release after 12 h (%) were evaluated as responses. Matrix of the design including investigated factors, responses and levels are also shown in Table 4. The mathematical model generated by three factors and two responses factorial design is following [()]:

$$Y_0 = b_0+b_1A+b_2B+b_3C+b_{12}AB+b_{13}AC+b_{23}BC+b_{23}BC+b_{11}A2+b_{22}B2+b_{33}C_2$$

Where Y is the response; b_0 is a constant; b_1,b_2 and b_3 are the coefficients translating the linear weight of A, B and C, respectively; b_{12} , b_{13} and b_{23} are the coefficients translating the interactions between the variables; and b_{11} , b_{22} and b_{33} of the coefficients translating the quadratic influence of A, B and C. Linear and second-order polynomials were fitted to the

experimental data to obtain the regression equations, and their observed and predicted responses are given in Table 11. One-way ANOVA was applied to estimate the significance of the model ($p < 0.05$).

Table: 4 Design of experiment-levels of various process parameters

	LEVEL USED		
FACTORS	LOW (-1)	MEDIUM (0)	HIGH (+1)
X_1 – Sodium alginate concentration (%w/v)	2	4	6
X_2 - OG concentration (%w/v)	2	4	6
X_3 – Cross linking concentration (%w/v)	5	10	15

Response variable: Y_1 – Drug loading (%); Y_2 – Drug release in 12HRS (%)

Preparation of Capecitabine-loaded Ca^{2+}-ion induced alginate-OG beads

Capecitabine -loaded alginate-OG blend beads were formulated by ionic-gelation cross-linking method using $CaCl_2$ as cross-linker in an aqueous environment. Briefly, SA and OG aqueous dispersions were prepared separately using distilled water and solutions of SA-OG blends were prepared. These solutions of SA-OG blends were well mixed with stirring for 10 min at 1000 rpm using a magnetic stirrer (Remi Motors, India).[61]

Afterwards, capecitabine was added to the single w/w emulsion of SA-OG blends. The ratio of drug to polymer was maintained 1:2 in all formulations and mixed thoroughly using a sonicator (Remi Motors, India).

The resulting dispersions were extruded through a 21-G flat-tipped hypodermic needle into slightly agitated 100 ml of aqueous $CaCl_2$ solutions containing 20% (w/v). Added droplets were retained in the $CaCl_2$ solutions for 15 min. The wet beads were collected by decantation, washed two times with distilled water and dried at 37°C in a hot air oven for overnight. The dried beads were stored in desiccators until used.

Preparation of enteric-coated OG beads[79]

The OG beads prepared above were transferred into ethanol solutions of Eudragit S 100 at various concentrations of 2.5, 5.0, and 7.5%, and coated for 30 min with stirring (500 rpm). The resulting coated-OG beads were filtered and air dried. This coating process was repeated five times.

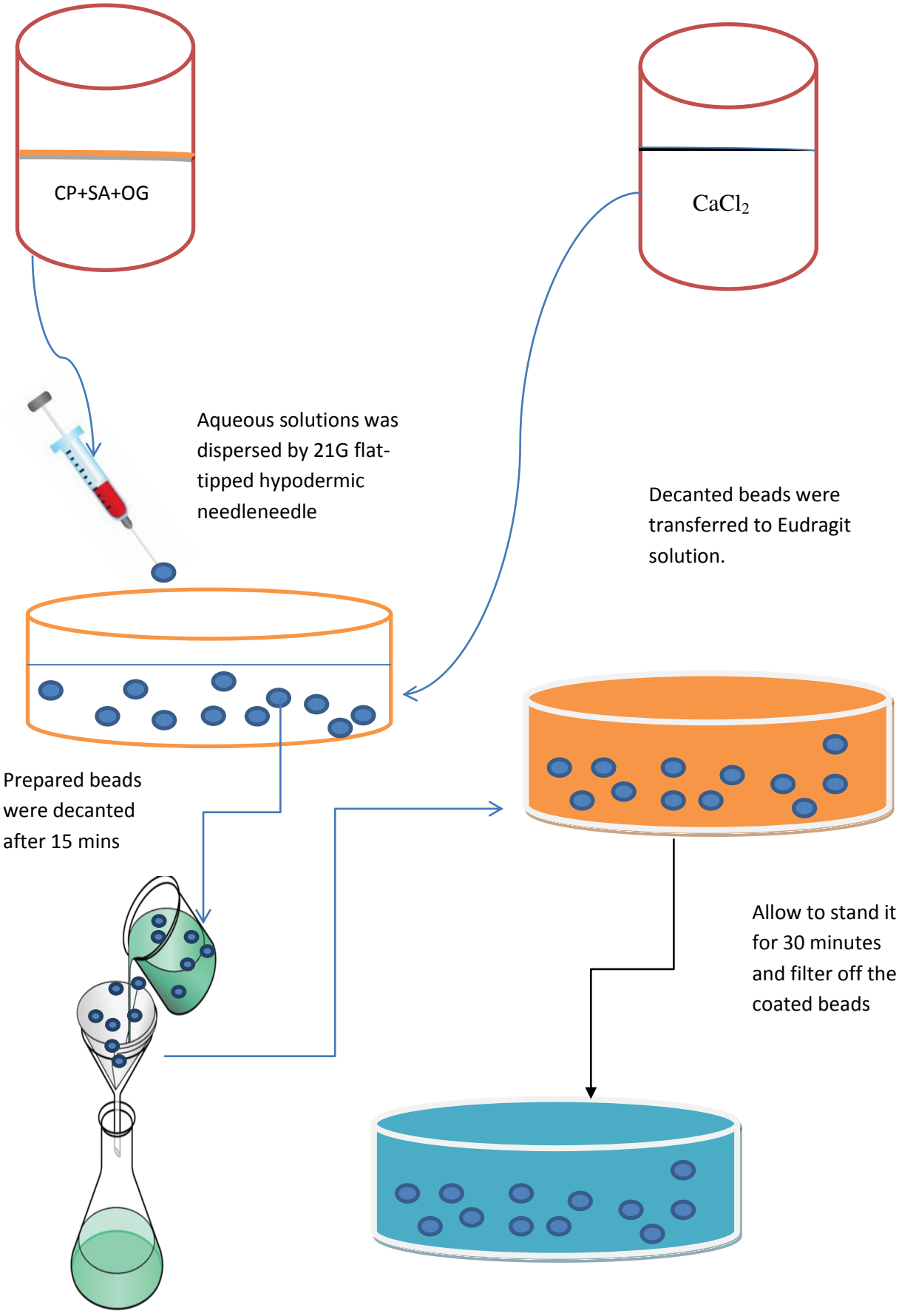

Figure: 7 Method of preparation of beads

Characterization of beads[61]

Determination of Drug Loading

Accurately weighed 100 mg of Capecitabine-loaded alginate-OG blend beads were taken separately and were crushed using pestle-mortar. The crushed powders were placed in 500 ml of phosphate buffer, pH 7.4, and kept it overnight followed by sonication for 15 min in a sonicator (Vibra Pvt. Ltd., India). The polymer debris formed after disintegration of beads was removed filtering through Whatman® filter paper (No. 40).The drug content in the filtrate was determined using a UV-VIS spectrophotometer (Shimadzu, Japan) at 276 nm. DEE of beads was calculated using this following formula:

$$DL = \frac{\text{Actual drug content in beads}}{\text{Theoretical drug content in beads}} \times 100.$$

Bead size measurement

The diameters of dried beads were measured using Veriner calipers having an accuracy of 0.001 mm. Dried beads were taken and inserted in between the space of two metallic plates. Diameters of resultant beads were displayed in the digital screen of the previously calibrated equipment. The average size was then calculated by measuring the diameter of 3 sets of 20 beads from each batch.

SEM analyses

The surface morphology of the formulated beads was analyzed by scanning electron microscope (SEM) (JEOL, Japan). Beads were gold coated by mounted on a brass stub using double-sided adhesive tape and under vacuum in an ion sputter with a thin layer of gold (3–5 nm) for 75 s. SEM photograph was taken at an acceleration voltage of 20 kV.

Differential scanning calorimetry (DSC)

The DSC thermograms of the pure drug, drug-loaded micro beads and polymers were obtained using the Perkin Elmer JADE DSC system, to identify any interaction between the components of formulations.

FTIR spectroscopy analyses

Samples were reduced to powder and analyzed as potassium bromide pellets by using an FTIR spectroscope (Perkin Elmer, USA).Each sample was mixed with potassium bromide at a ratio of 1:9 and converted into pellet at 100 kg pressure using a hydraulic press pellet

technique. Beads were placed in the sample holder and spectral scanning was taken in the wavelength region between 3800 and400 cm^{-1}.

Evaluation of swelling behavior

Swelling behavior evaluation of Capecitabine-loaded alginate-OG blend beads was carried out in 0.1 N HCL (pH 1.2) and phosphate buffer(pH 7.4). 100 mg beads were placed in vessels of dissolution apparatus (Campbell Electronics, India) containing 500 ml respective media. The experiment was carried out at 37 ± 1°C under 50 rpm paddle speed. At pre-determined time intervals, swelled beads were taken out from the solution using stainless steel grid; excess surface liquid was removed gently without pressing hard and the weight was recorded using electronic microbalance (Model BL-220H, Shimadzu, Japan). Swelling index was determined using the following formula:

$$\text{Swelling index } (\%) = \frac{\text{Weight of beads after swelling} - \text{Dry weight of beads}}{\text{Dry weight of beads}} \times 100$$

In vitro drug release study

The in vitro release of Capecitabine-loaded alginate-OG blend beads was tested using a dissolution apparatus USP/BP/IP (Campbell Electronics, India). The baskets were covered with 100-mesh nylon cloth to prevent the escape of the beads. The dissolution was measured at37 ± 1°C under 50 rpm speed. Accurately weighed quantities of Capecitabine-loaded OG-alginate beads equivalent to 100 mg Capecitabine were added to900 ml of 0.1 N HCL (pH 1.2). The test was carried out in 0.1 N HCL for 2 h, and then, continued in phosphate buffer (pH 7.4) for next6 Hrs. 5 ml of aliquots were collected at regular time intervals, and the same amounts of fresh dissolution medium were replaced into dissolution vessel. The collected aliquots were filtered, and suitably diluted to determine the absorbance using a UV-VIS spectrophotometer (Shimadzu, Japan) at 276 nm.

Analysis of in vitro drug release kinetics and mechanism

The in vitro drug release data were evaluated kinetically in important mathematical models:

Zero-order model: $Q = kt + Q^0$;

First-order model: $Q = Q_0 e^{k.t}$;

Hixson–Crowell model: $Q^{1/3} = kt + Q_0^{1/3}$;

Higuchi model: $Q = kt^{0.5}$.

Korsmeyer–Peppas model: $Q = kt^{n}$;

where Q = drug released amount in time t, Q_0= start value of Q; k = rate constant, a = time constant, b = shape parameter and n = release exponent, indicative of drug release mechanism. The accuracy of these models was compared by calculation of squared correlation coefficient (R_2) using Kinet DS 3.0 Rev. 2010 software. Again, the Korsmeyer–Peppas model was employed in the in vitro drug release behavior analysis of these formulations to distinguish between competing release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous transport), and case-II transport(relaxation-controlled release). When n is ≤0.43, it is Fickian release. The n-value between 0.43 and 0.85 is defined as non-Fickian release. When, n is ≥0.85, it is case-II transport.

Statistical analysis

Statistical optimization was performed using Design-Expert10 software (Stat-Ease Inc., USA). All measured data are expressed as mean ± standard deviation (S.D.) and analyzed using Bio Stat version 10 2009 for Windows software.

RESULT AND DISCUSSION

Characterization of Okra gum [61]

After extraction and further precipitation by acetone the yield of mucilage was 50.44% w/w obtained. The isolated sample was subjected to identification. The results for loss of drying showed value of 9.917%. This indicated that mucilage is hygroscopic in nature and need to be stored in air-tight containers. In solubility behavior of okra mucilage was found to be soluble in warm water, slightly soluble in cold water and insoluble in benzene, ether, chloroform, n-butanol, ethanol, acetone, glycerin, paraffin. Surface tension of 0.25% w/v solutions of mucilage was found to be 0.0405 joule/m. Other phyto-constituents were absent in the 2 isolated powder, pH of 1% solution was found to be 7.5. Irregular particles size was found to be 52.50µm. Result obtained of okra mucilage and observed that mucilage is brownish color, odorless, tasteless, rough and irregular in shape. Ash values were calculated to characterize mucilage; total ash, acid insoluble ash and water soluble ash were found 7.53%, 0.93% and 4% respectively. Physical characterization of mucilage was carried out for bulk density and bulkiness, true density, total porosity, powder flow behaviour. The bulkiness value indicated that powder is 'heavy' in nature. Result obtained in micromeritic characterization of mucilage was shown in Table 5.

Table: 5 Micromeritic study data of mucilage

S. No	Parameters	Values
1	Angle of repose (º)	26.22
2	Carr's index (%)	74.68
3	True density (gm/ml)	2.74
4	Bulk density (gm/ml)	0.637
5	Bulkiness (ml/g)	1.34
6	Mean particle size (µ)	54.41

Characterization of Capecitabine

Table: 6Physical properties of capecitabine

S. No	Description	Result
1.	Colour	White
2.	Odor	Odorless
3.	Appearance	Powder
4.	Melting point	116-117ºC
5.	Solubility	Soluble in water, ethanol, >60% dimethyl form amide, marginally soluble in >40% methanol

Standard curve of Capecitabine in 0.1N HCL, 5.8 phosphate buffer, 7.4 phosphate buffer

Table: 7 Calibration curve of Capecitabine in 0.1N HCL

Concentration (mcg/ml)	Absorbance (nm)
0	0
10	0.165
20	0.332
30	0.49
40	0.663
50	0.823
60	0.997

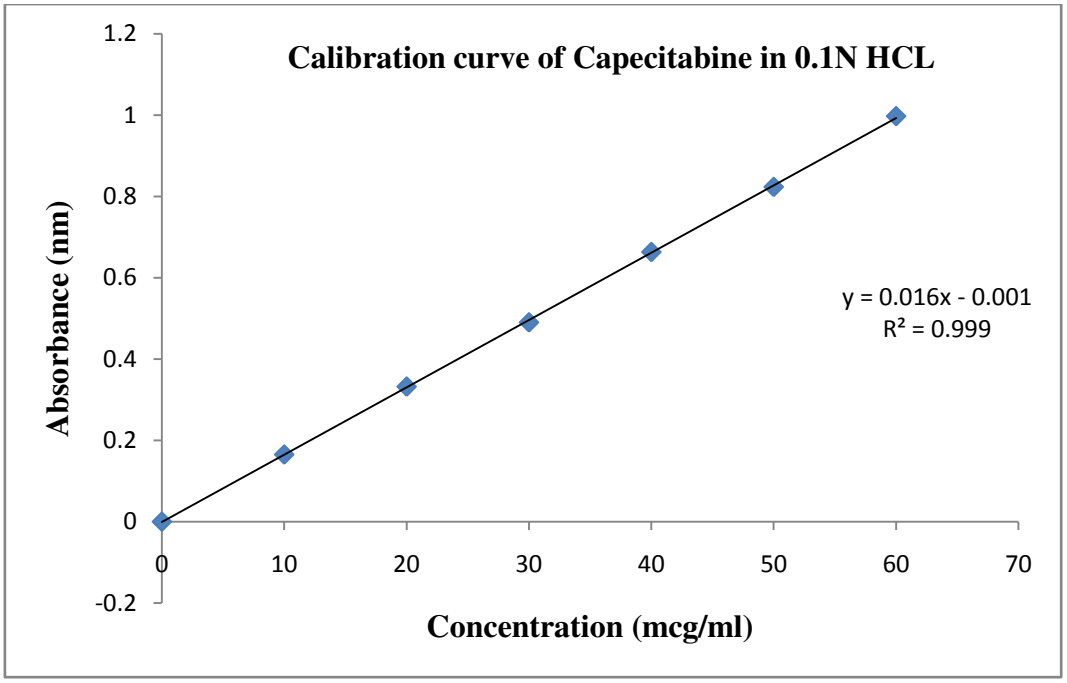

Figure: 8Calibration curve of Capecitabine in 0.1N HCL

Table: 8 Calibration curve of Capecitabine in 5.8 phosphate buffer

Concentration (mcg/ml)	Absorbance (nm)
0	0
10	0.468
12	0.568
14	0.662
16	0.765
18	0.862
20	0.961

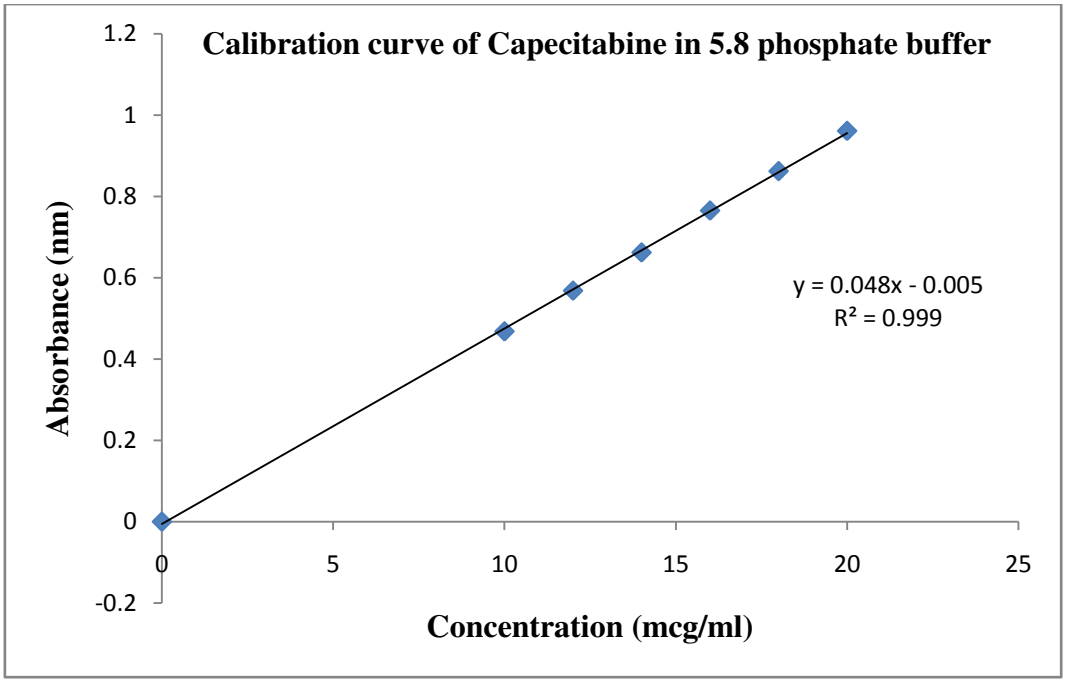

Figure: 9 Calibration curve of Capecitabine in 5.8 phosphate buffer

Table: 9 Calibration curve of Capecitabine in 7.4 phosphate buffer

Concentration (mcg/ml)	Absorbance (nm)
0	0
10	0.404
12	0.488
14	0.572
16	0.655
18	0.748
20	0.826

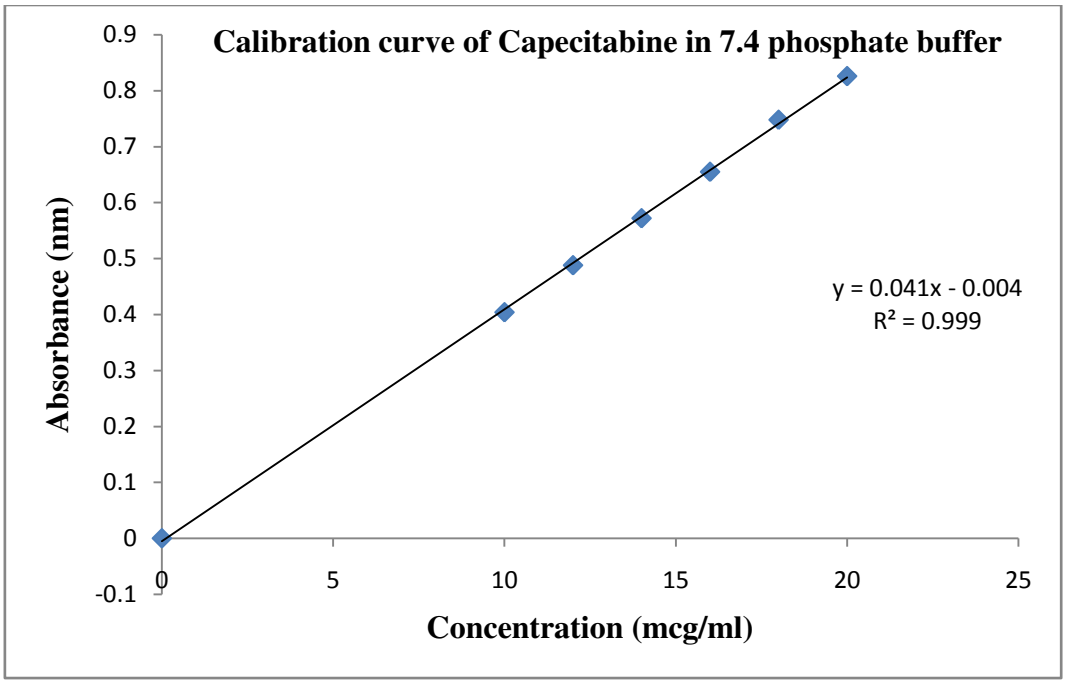

Figure: 10Calibration curve of Capecitabine in 7.4 phosphate buffer

Optimization of CP-loaded alginate-OG blend beads[80]

According to Box – Behnken factorial design, various Capecitabine loaded alginate okra blended beads were prepared using Ca^{2+} ion using ionic gelation cross linking method. The matrix of the design including three factors (sodium alginate concentration, OG concentration, cross linking concentration) and two responses (drug loading, drug release) represented in Table 10. The seventeen trial runs with respect to value responses were fitted into the design to get the model equation for responses. The results suggested that two quadratic equations involving individual main factors and interaction factors. The results of experimental design shown in Table 10 and it indicates that this model were significant for all response parameters. The model equation for the response Y_1 (drug loading) and response Y_2 (drug release) is shown below.

Y_1=64.00+6.31A+10.67B+2.99C-7.19A^2-8.16B^2-3.84C^2-0.15AB-0.025AC+1.50BC

Y_2=97.40+15.00A+0.50B-8.00C-15.08A^2-19.58B^2-12.07C^2-2.75AB+7.75AC-1.75BC

Where A, B and C were represent the polymer concentration, amount of cross linking agent and drug concentration respectively. A positive value represents an effect that favours the optimization, while a negative value indicates an antagonistic effect. The values of A, B, and C were substituted in the equation to obtain the theoretical values of Y_1. The predicted values and the observed values were found to be in good agreement. The effect of pair wise interaction of the parameters is depicted in the three dimensional graphs when the third parameter is kept constant. The optimum condition for the preparation of Capecitabine beads as evident from Figure 11 are 4% w/v of sodium alginate concentration, amount of cross linking agent 10% w/v and 4%w/v OG concentration.

Therefore the optimum procedure was determined to be the owing Briefly, SA and OG aqueous dispersions were prepared separately using distilled water and solutions of SA-OG blends were prepared. These solutions of SA-OG blends were well mixed with stirring for 10 min at 1000 rpm using a magnetic stirrer (Remi Motors, India). Afterwards, Capecitabine was added to the single w/w emulsion of SA-OG blends. The ratio of drug to polymer was maintained 1:2 in all formulations and mixed thoroughly using a homogenizer (Remi Motors, India). The resulting dispersions were extruded through a 21-G flat-tipped hypodermic needle into slightly agitated 100 ml of aqueous $CaCl_2$ solutions containing 10% w/v. Added droplets were retained in the $CaCl_2$ solutions for 15 min. The wet beads were collected by decantation, washed two times with distilled water and dried at 37°C in a hot air oven for overnight. The dried beads were stored in desiccators until used.

Table: 10Experimental runs and observed values of responses for Box-Behnken design

		INDEPENDENT VARIABLES			DEPENDENT VARIABLES			
					ACTUAL		PREDICTED	
RUNS	BATCH	A	B	C	Y_1	Y_2	Y_1	Y_2
1.	F1	0	0	0	63.52	96.89	64.02	97.37
2.	F2	-1	1	0	54.81	51.60	55.16	51.23
3.	F3	1	-1	0	43.03	80.74	42.61	80.17
4.	F4	1	0	1	62.18	89.59	62.93	89.11
5.	F5	-1	0	1	48.45	41.03	48.35	40.45
6.	F6	1	1	0	64.72	72.46	65.28	72.15
7.	F7	0	1	-1	58.18	81.05	57.74	80.63
8.	F8	-1	0	-1	43.64	67.13	43.23	67.76
9.	F9	1	0	-1	57.88	84.50	58.65	85.12
10.	F10	-1	-1	0	32.42	48.11	32.84	48.69
11.	F11	0	0	0	63.52	96.89	64.02	97.37
12.	F12	0	0	0	63.52	96.89	64.02	97.37
13.	F13	0	1	1	66.67	56.68	67.37	56.14
14.	F14	0	0	0	63.52	96.89	64.02	97.37
15.	F15	0	0	0	63.52	96.89	64.02	97.37
16.	F16	0	-1	1	44.75	56.31	44.05	55.62
17.	F17	0	-1	-1	39.92	71.84	40.46	72.49

Response variable: Y_1 – Drug loading (%); Y_2 – Drug release in 12HRS (%)

The influences of independable factors on dependable responses investigated (here, DL, and DR12h) were further elucidated and analyzed by response surface methodology (RSM). RSM is an alternate statistical approach, which is able to represent the effect of interaction between different independable factors. The main advantages of the RSM are the reduced numbers of experimental trials needed to evaluate multiple parameters and their interactions and it is

useful for developing, improving and optimizing process.The 3-dimensional response surface plot relating DL depicts increasing the values of OG (B) as well as decreasing the values of SA (A) and increasing $CaCl_2$ concentration (C) (Figs. 11, 12 and 13 respectively). However, an increase in DR12hvalues with the increasing OG(B) and decreasing SA(A) and increasing $CaCl_2$ concentration (C) is indicated by the 3-dimensional response surface plot relating DL and DR12h (Figs. 14, 15 and 16, respectively).

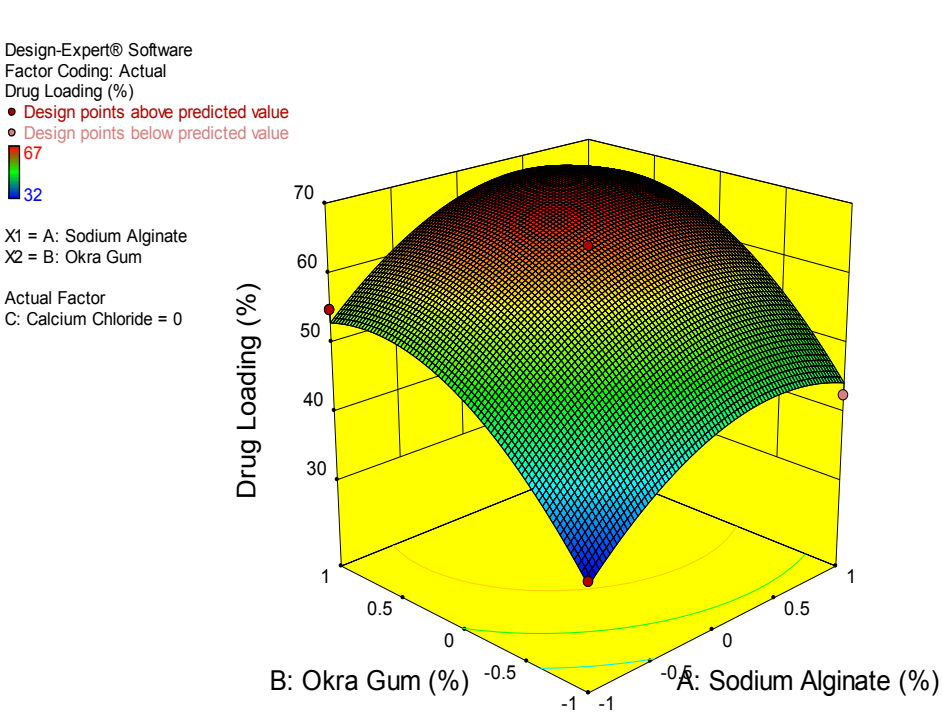

Figure:11 Three dimensional response surface plots relating % drug loading between okra gum and sodium alginate

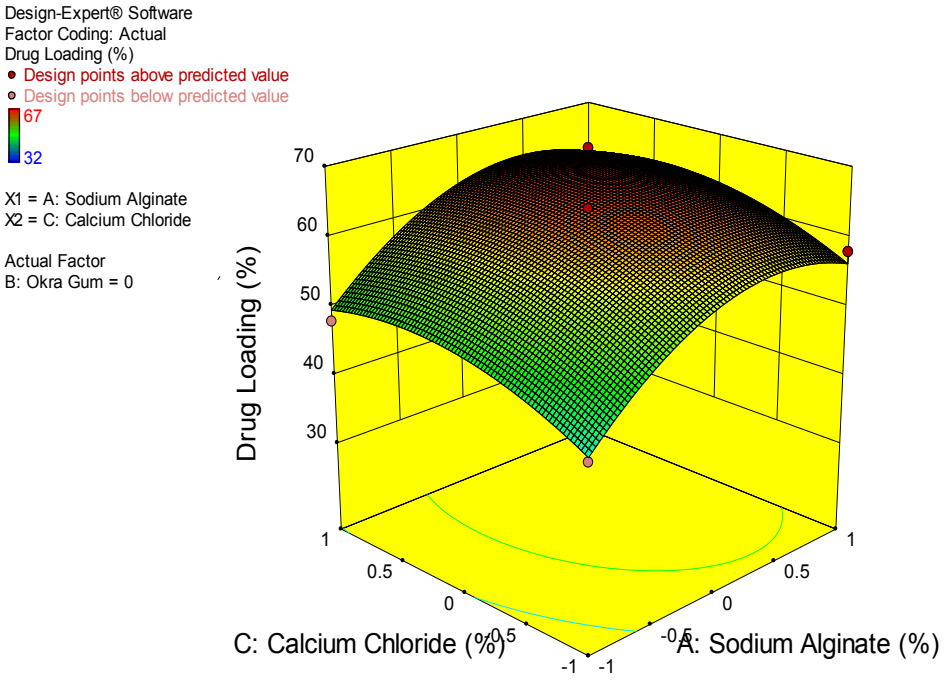

Figure: 12 Three dimensional response surface plots relating % drug loading between Calcium chloride and sodium alginate

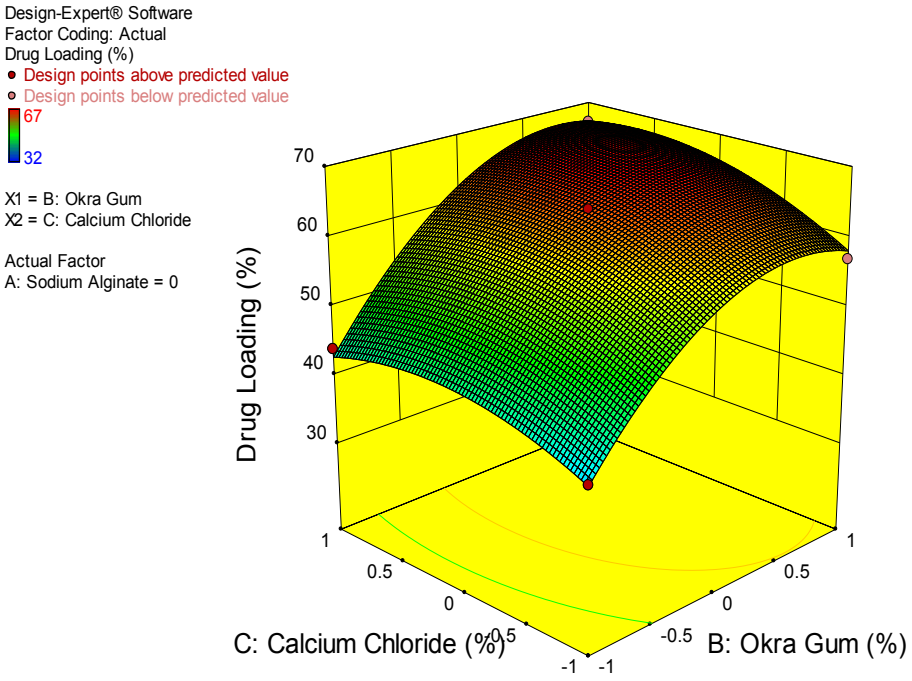

Figure: 13 Three dimensional response surface plots relating % drug loading between okra gum and Calcium chloride

To develop a new formulation with the desired responses, numerical optimization technique was employed using the desirability approach. To get the desired optimum responses for CP-loaded alginate-OG blend beads, factors were restricted to$2 \leq A \leq 6\%$, $2 \leq B \leq 6\%$ and $5 \leq C \leq 15\%$whereas the desirable ranges of responses were restricted to $32 \leq DL \leq 67(\%)$ and $40 \leq DR12h(\%) \leq 97\%$under comprehensive evaluation of the feasibility search and subsequently exhaustive grid searches.

The optimal values of responses were obtained by numerical analysis using the Design-Expert 10.0.6.1 software based on the criterion of desirability. The overlay plot indicating the region of optimal process variable settings was presented in Fig. 11-16. In order to evaluate the optimization capability of these models generated according tothe results of 3^2-factorial design, optimized Ca^{2+}-ion induced CP-loaded alginate-OG blend beads were prepared by ionic-gelation cross-linking were prepared using one of the optimal process variable settings proposed by the design. The selected optimal process variable setting used for the formulation of optimized CP-loaded alginate-OG blend beads. Table 10 lists the results of experiments with predicted responses by the mathematical models and those actually observed. The optimized CP-loaded alginate-OG blend beads (F-1) showed DL of$64.02 \pm 1.58\%$ and DR12h of $97.32 \pm 2.83\%$ with small error-values(-1.82 and 2.85, respectively).

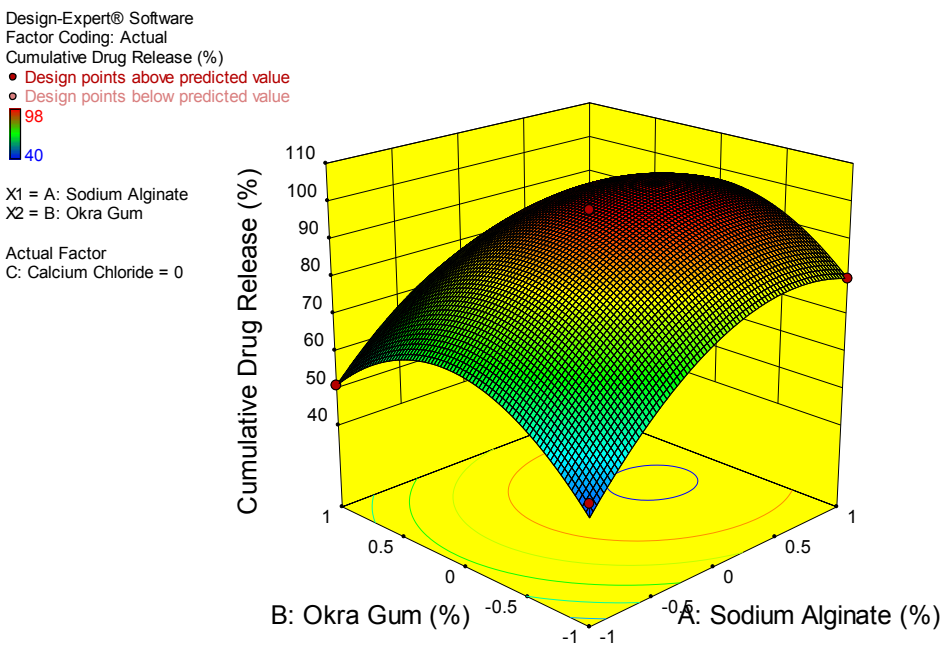

Figure: 14Three dimensional response surface plots relating % drug release between okra gum and sodium alginate

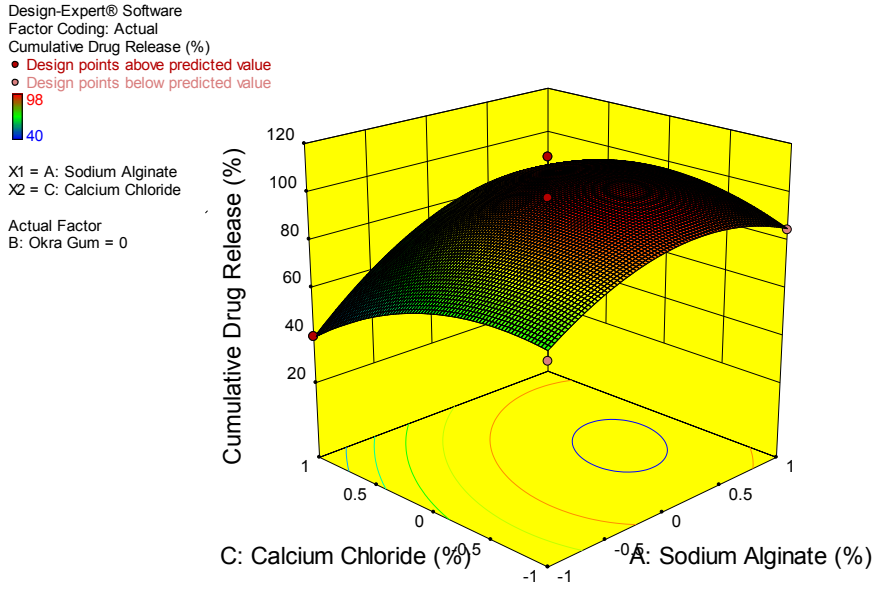

Figure: 15 Three dimensional response surface plots relating % drug release between calcium chloride and sodium alginate

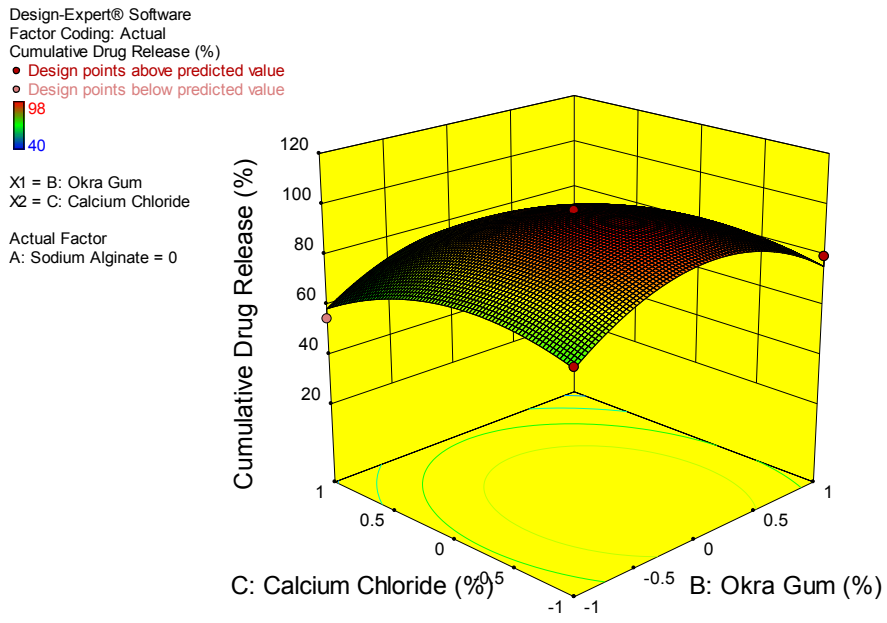

Figure: 16 Three dimensional response surface plots relating % drug release between calcium chloride and Okra gum

Table: 11Regression analysis for response Y_1 and Y_2

Model	R-squared	Adjusted R-squared	Predicted R-squared	SD	Remarks
Response (Y_1)					
1. Linear	0.6688	0.5924	0.2611	7.04	-
2. Second order	0.6735	0.4776	-0.1316	7.97	-
3. Quadratic	0.9915	0.9806	-1.3871	1.54	Suggested
4. Cubic	1.0000	1.0000	N/A	8.944	-
Response (Y_2)					
1. Linear	0.3717	0.2267	0.0593	17.35	-
2. Second order	0.4171	0.0673	-0.4376	19.05	-
3. Quadratic	0.9854	0.9667	0.7697	3.60	Suggested
4. Cubic	0.9998	0.9992	N/A	0.55	-

Table: 12 Summary of ANOVA for response parameters

Source	Sum of square	d.f	Mean square	F value	p-value Prob>F
For D.L (%)					
Model	1929.75	9	214.42	90.85	<0.0001
A	318.91	1	318.91	135.12	<0.0001
B	911.43	1	911.43	386.17	<0.0001
C	71.40	1	71.40	30.25	0.0009
AB	0.093	1	0.093	0.039	0.8483
AC	2.500	1	2.500	1.059	0.9749
BC	9.00	1	9.00	3.81	0.0918
A^2	217.56	1	217.56	92.18	<0.0001
B^2	280.58	1	280.58	118.88	<0.0001
C^2	62.11	1	62.11	26.32	0.0014

For D.R (%)					
Model	6135.42	9	681.71	52.61	<0.0001
A	1800.00	1	1800.00	138.92	<0.0001
B	2.00	1	2.00	0.15	0.7061
C	512.00	1	512.00	39.51	0.0004
AB	30.25	1	30.25	2.33	0.1704
AC	240.25	1	240.25	18.54	0.0035
BC	12.25	1	12.25	0.95	0.3633
A^2	956.87	1	956.87	73.85	<0.0001
B^2	1613.39	1	1613.39	124.52	<0.0001
C^2	613.92	1	613.92	47.38	0.0002

DRUG LOADING

Drug loading of Capecitabine -loaded alginate-OG blend beads was found 67%. It was found that the drug loading in these beads was increased with decreasing concentration of SA (A)and increasing the concentration of OG (B) and increasing concentration of $CaCl_2$(C) in cross-linking solutions. Increased drug loading with increasing OG might be due to increase in viscosity of the polymer-blend solutions with decreasing concentration of SA. This could have been prevented drug leakage to the cross-linking solution. Again, the drug loading in these beads was increased with increasing $CaCl_2$ concentration in cross-linking solutions and this could be due to high degree of cross-linking. The high degree of Ca^{2+}-ion induced cross-linking of alginate forms insoluble dense matrices, which results more drug loading in these beads. The Capecitabine -loaded alginate-OG blend beads prepared using lower $CaCl_2$ concentration might have larger pores due to insufficient cross-linking and/or drug leaching through the pores. The insufficient cross-linking and/or drug leaching through the pores could result in lower drug loading. [85]

BEADS SIZE [83]

The average bead diameter of Capecitabine-loaded alginate-OG blend beads was within the range of 1.016mm. Increase in the average size of these beads was found with the decreasing of SA. This could be attributed due to the increase in viscosity of polymer-blend solution with incorporation of increasing OG that in turn increased the droplet size of polymer-blend solutions to the cross-linking solutions during preparation. With the increasing amount of OG in the polymer-blends, the number of free sites available for cross-linking could be less so that the bead sizes with decreasing SA. The decrease in bead size of Capecitabine-loaded alginate-OG blend beads was observed, when concentrated $CaCl_2$ solutions were used for cross-linking. This could be due to shrinkage of polymeric-gel by higher degree of cross-linking. The shrink-age of the alginate-containing polymeric gel under the influence of concentrated $CaCl_2$ solutions can be attributed that the Ca^{2+}-ions penetrate the interior of alginate-OG blend droplets and water is squeezed out of the interior of droplets, which results shrinkage of beads. When the polymer concentration increased, the release percentage increases initially followed by decrease in drug release. The low release of the drug from the beads at higher polymer concentration may be due to delayed swelling of the beads with larger particle size formation.

SURFACE MORPHOLOGY

The surface morphology of Capecitabine-loaded alginate-OG blend beads was visualized by SEM and is presented in Fig. 17-19. The surfaces of these beads were appeared to have rough with characteristic large wrinkles and cracks, as it was evident from the SEM photographs. This might be caused by partly collapsing the polymeric gel network during drying. Moreover, few polymeric derbies and drug crystals were seen on the bead surface. Presence of polymeric derbies on the bead surface could be due to the simultaneous gel bead preparation and formation of the polymer blend matrix; whereas the presence of drug crystals on the bead surface might be formed as a result of their migration along with water to the surface during drying.

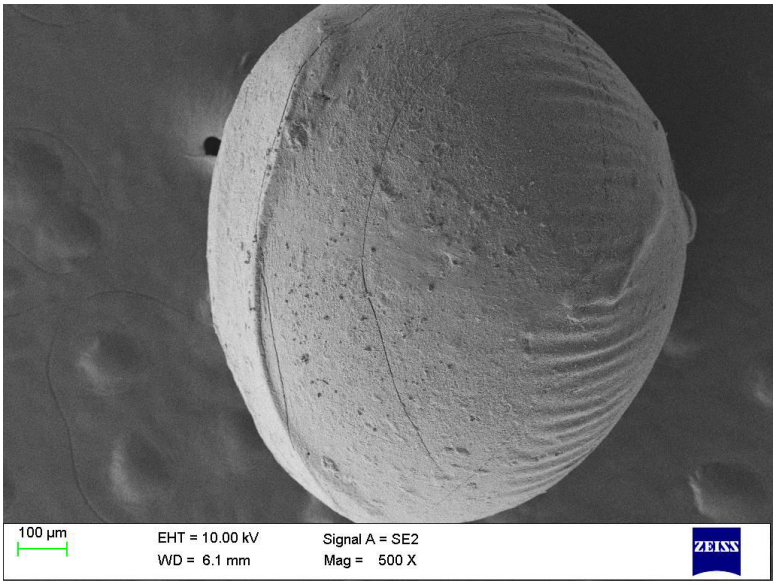

Figure: 17 SEM of Capecitabine loaded alginate- okra gum beads

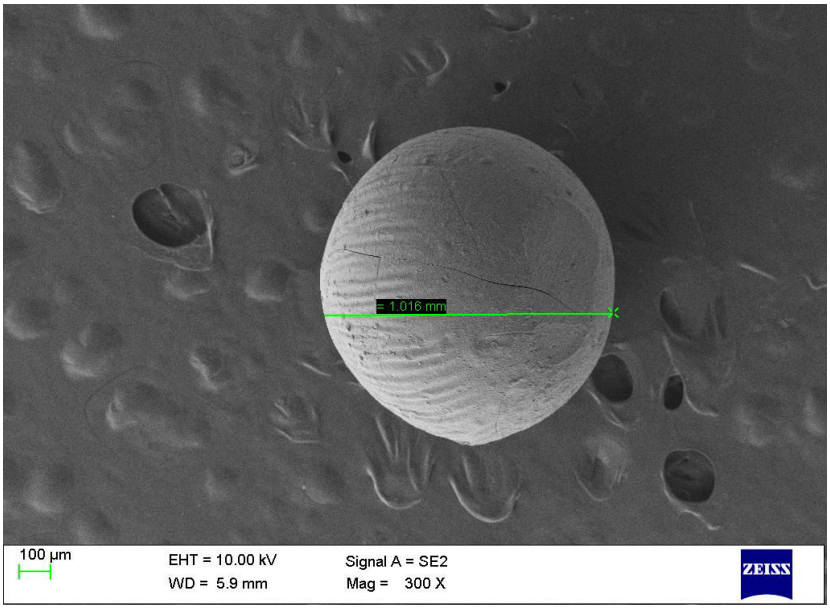

Figure: 18 SEM of Eudragit coated Capecitabine loaded alginate –okra gum beads

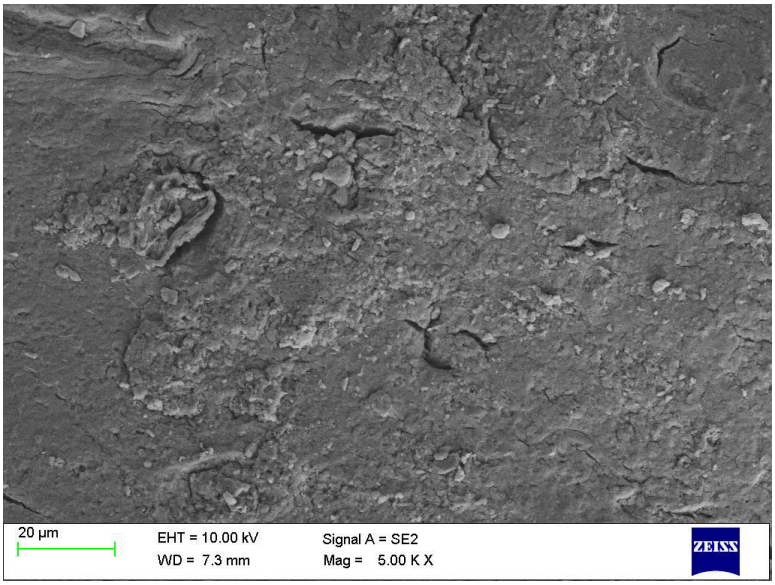

Figure: 19 SEM analyses shows the surface is porous and wrinkled

FT-IR

The FTIR spectra of SA, Eudragit, isolated OG, optimized Capecitabine-loaded alginate-OG blend beads and pure Capecitabine are shown in Fig.20 to 24.

In the FTIR spectrum of SA, the characteristic peaks were appeared 1416 cm^{-1} and 1616 cm^{-1}, for symmetric and asymmetric –C=O stretching vibrations of -COO^-anions, respectively. In addition, a wideband at 3441 $cm^{-1}$was appeared due to the -OH stretching vibrations Fig.21.

The spectrum of isolated OG showed an identical small peak at 1411 cm^{-1}due to –C-H bend, a small peak at 1726 cm^{-1}due to –C=O stretch, peak at 2928 cm^{-1}due to –C- H stretch and a broad band at 3419 cm^{-1}due to -OH stretching vibrations Fig. 22.

FTIR spectrum of Eudragit S-100 showed the peak at 2953.9 cm^{-1} due to presence of O–H (carboxylic acid), at 1450.7 cm^{-1} due to -CH_3 bend, and at 1731.2 cm^{-1} due to the presence of C = O (ester).

In the spectrum of Capecitabine, there are sharp peaks at 1716, 1502, 3215, and 3520 cm^{-1} corresponding to C=O stretching vibrations (pyrimidine carbonyl), N=O bending vibrations, N-H bending vibrations (tetra hydro furan), and -OH stretching vibrations respectively. These peaks were found in drug-loaded Eudragit coated -sodium alginate OG

beads. This confirms that no chemical interaction was found between the drug and polymers, thus confirming drug compatibility with these excipients.

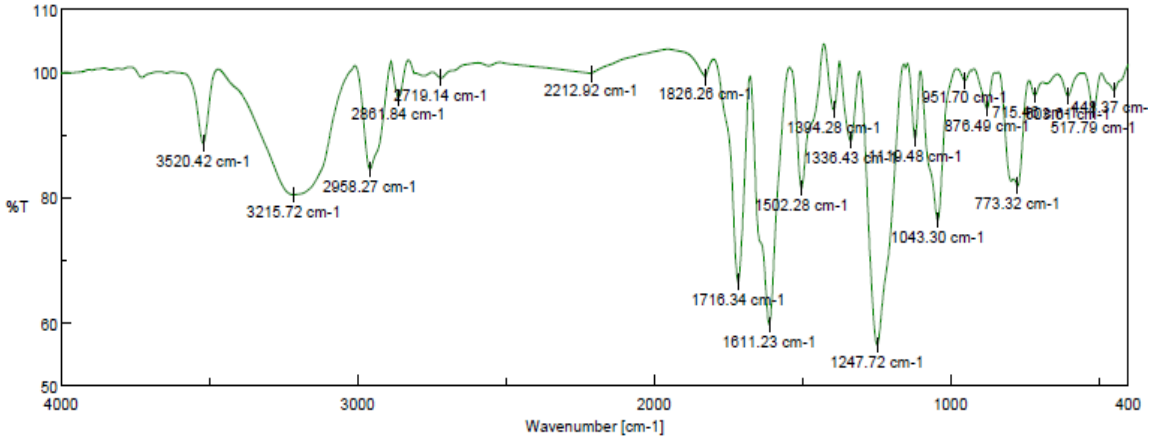

Figure: 20 FT-IR Spectra of Capecitabine Drug

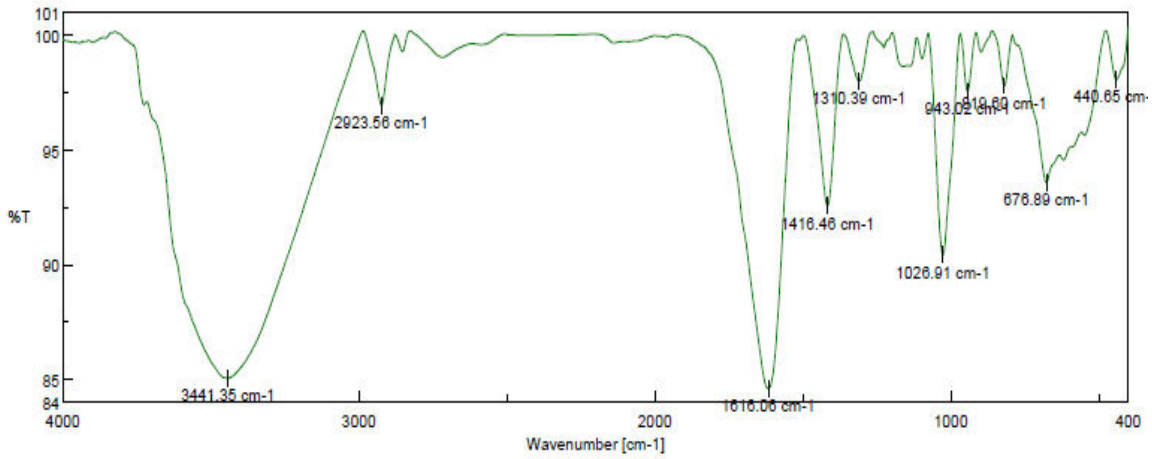

Figure: 21 FT-IR Spectra of Alginate

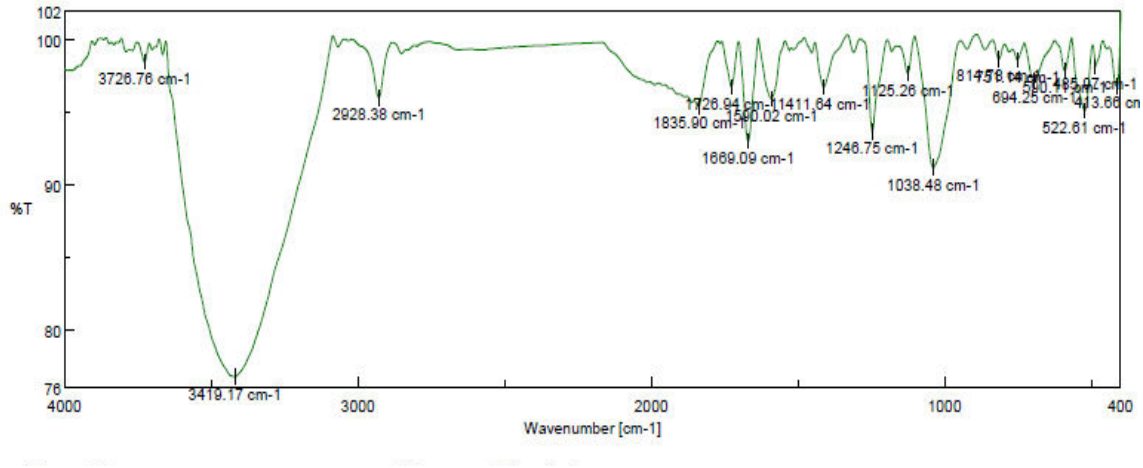

Figure: 22 FT-IR Spectra of Okra gum

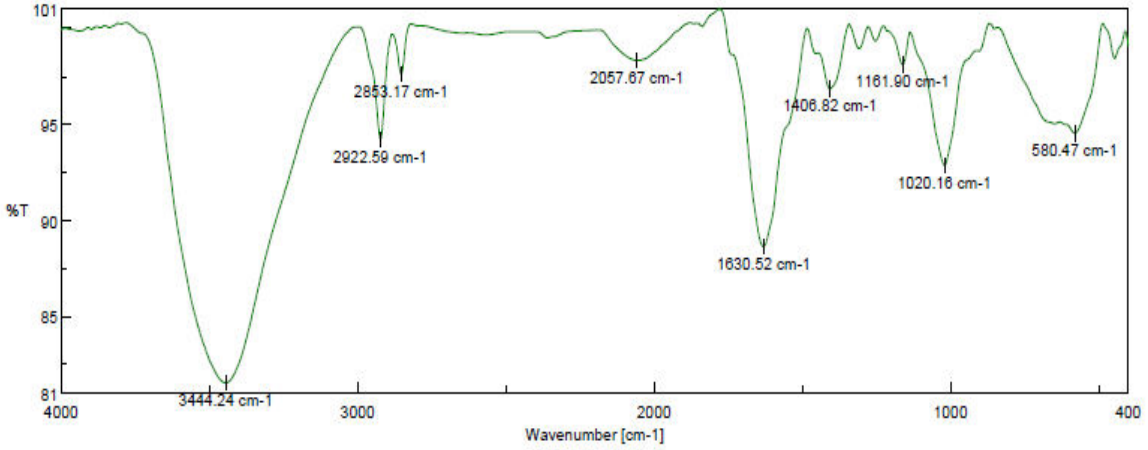

Figure: 23 FT-IR Spectra of Eudragit S 100

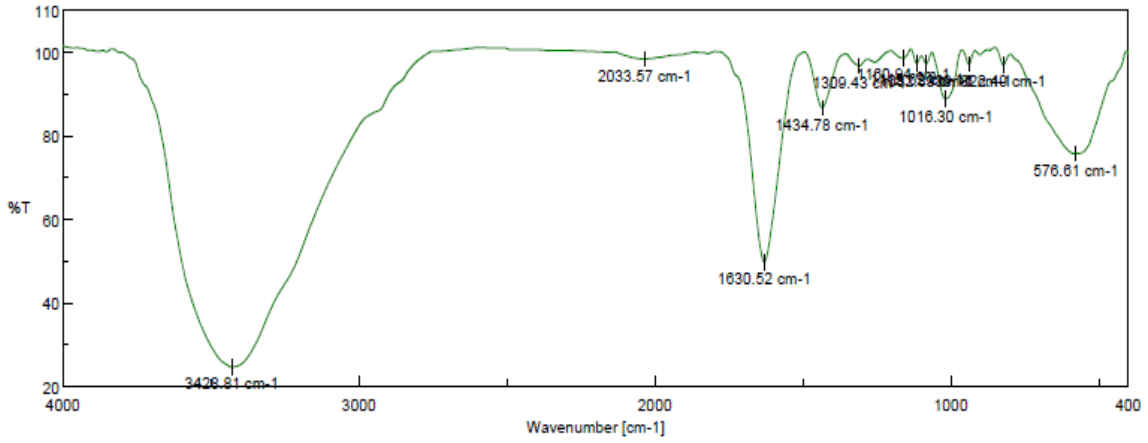

Figure: 24 FT-IR Spectra of Eudragit Coated alginate OG beads

SWELLING STUDIES

The swelling behavior of optimized Ca^{2+}-ion induced CP-loaded alginate-OG blend beads (F-1) was evaluated in both acidic pH(0.1 N HCl, pH 1.2) and alkaline pH (phosphate buffer, pH 7.4). The results of swelling are shown in Fig. 25. Initially, the swelling index of OG-alginate beads (F-1) was lower in acidic pH in comparison with that of in alkaline pH. Under acidic pH, swelling of ionically gelled alginate-based beads occurs narrowly, which could probably due to formation of insoluble alginic acid regions through proton-calcium ion exchange and followed by solvent penetration into the gel-network. Maximum swelling was noticed at 3 h in alkaline pH and after which, erosion and dissolution of beads observed. The swelling behavior of optimized Ca^{2+}-ion induced CP-loaded alginate-OG blend beads (F-1) in alkaline pH could be explained by the ion exchanging between Ca^{2+}-ions of the Ca^{2+}-ion induced CP-loaded alginate-OG blend beads and the sodium ions present in phosphate buffer, with the influence of calcium sequestrant phosphate ions. This could result disaggregation of OG-alginate matrix structure leading to matrix erosion and dissolution of the swollen beads. The slow erosion of these beads could take place through slight degradation of polymeric-backbone into smaller molecular weight components. The overall swelling results suggested that the pH-sensitive swelling properties of these alginate-OG blend beads might be suitable for intestinal drug delivery as these beads were found to get rapidly dissolved in the higher pH prevailing in the intestine.

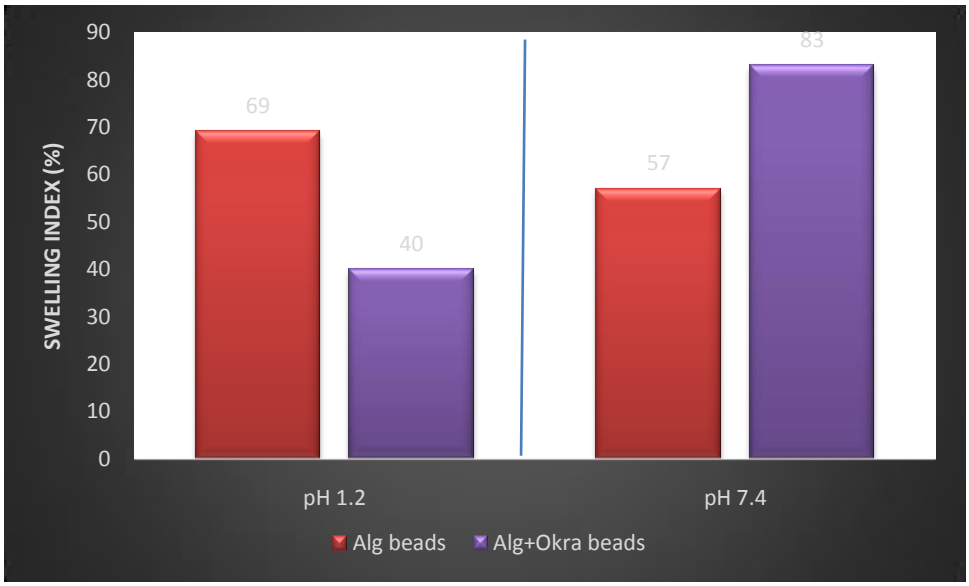

Figure: 25Swelling studies of beads in graphical representation

Differential Scanning Calorimetry (DSC)

The DSC thermogram of Capecitabine, drug loaded beads and polymers are shown in figure number 26(a) and 26(b). A sharp endothermic peak at 122.02º C was observed for pure Capecitabine corresponding to its melting point. There was no significant difference in thermogram of pure drug and drug loaded beads suggesting that, there is no interaction between drug and polymer.

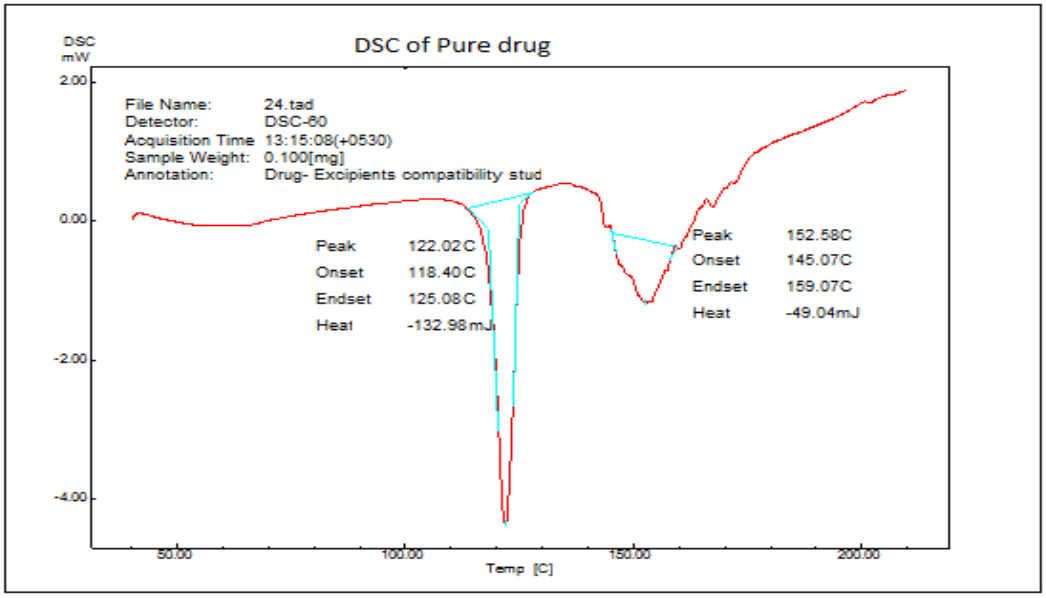

Figure: 26(a) Differential Scanning Calorimetry of Capecitabine

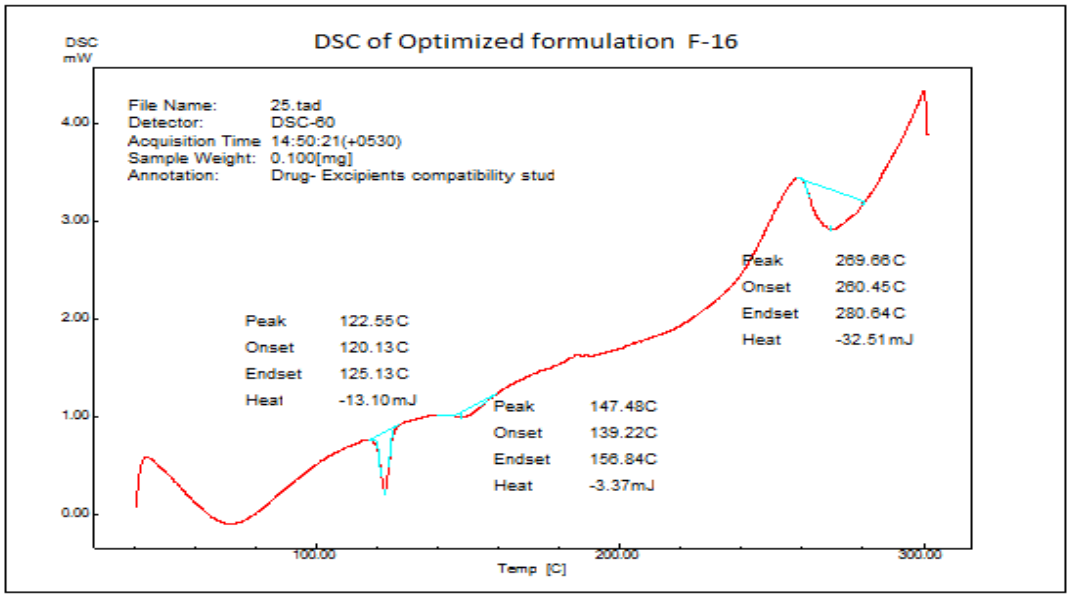

Figure: 26(b) Differential Scanning Calorimetry of CP loaded Alginate OG blend beads

IN-VITRO DRUG RELEASE[82]

Various Ca^{2+}-ion induced CP-loaded alginate-OG blend beads (F-1 to F-13) showed prolonged in vitro CP release over 12 h(Fig. 27).The trace amount of drug (CP) release from these alginate-OG blend beads at this preliminary stage of the in vitro drug release study could probably be due to the surface adhered drug crystals of CP, which were evidenced in SEM photograph in bead surface morphology analysis (Fig. 19).Amounts of CP released from OG-alginate beads in the acidic pH (1.2) up to 2 h were less and might be due tothe shrinkage of alginate at acidic pH (as alginate is pH sensitive)and poor solubility of alginate in lower pH, which could slower the drug release from OG-alginate beads. After that CP release from OG-alginate beads was observed faster in alkaline pH (7.4), which might be due to the higher swelling rate of these beads in alkaline pH. In case of beads containing higher OG amount, the more hydrophilic property of polymer-blends could bind better with water to form viscous gel structure, which may block the pores on bead surfaces and sustain the drug release from the Ca^{2+}-ion induced CP-loaded alginate-OG blend beads. The invitro drug release data reveals that drug release was observed in the acidic pH. In order to inhibit the drug release in acidic medium and target the drug release to colon the optimized batch of CP-loaded alginate OG blended beads (F1) were enteric coated with 2.5%,5% and 7.5% (F14, F15and F16) of Eudragit S-100 and further to the coated beads the invitro release was seen in both acidic and alkaline medium. The data reveals that there was no drug release in acidic medium (upto 2hrs). The result indicates that drug was protected completely from upper GIT condition by a coating of Eudragit S100, because Eudragit polymer contain carboxyl group that ionize in an environment where pH is greater than 7. As ionization take place, integrity of film was disturbed and drug is released. At pH 7.4, membrane coating get dissolved and beads were exposed to dissolution media following which the polymer matrix swells and erodes releasing entrapped drug. In the case of uncoated OG beads, although suppressed under highly acidic condition due to the low solubility of the non-ionized form of Capecitabine, which exists at this pH (pKa of Capecitabine, 4.5), and the consequent partitioning of the drug into the polysaccharide chains of the gels, a more significant release of Capecitabine occurred in comparison with Eudragit-coated OG beads (Kawasaki et al., 1999). However, at pH 7.4, Capecitabine was fully ionized and had a greater tendency to dissolve into the release medium. The higher release from the uncoated OG beads reflects the lower diffusion resistance of these core beads compared with that of the coated beads caused by the absence of a barrier against drug diffusion.

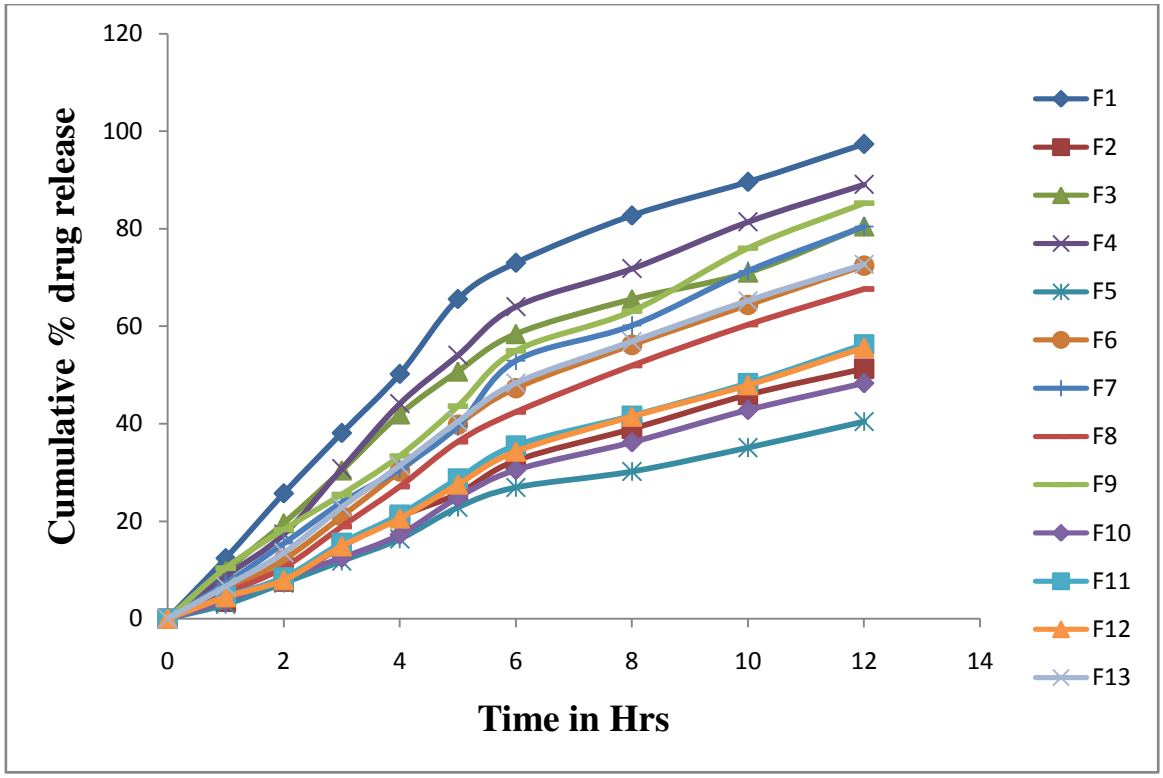

Figure: 27 Invitro- drug release study of CP loaded Alginate OG blend beads

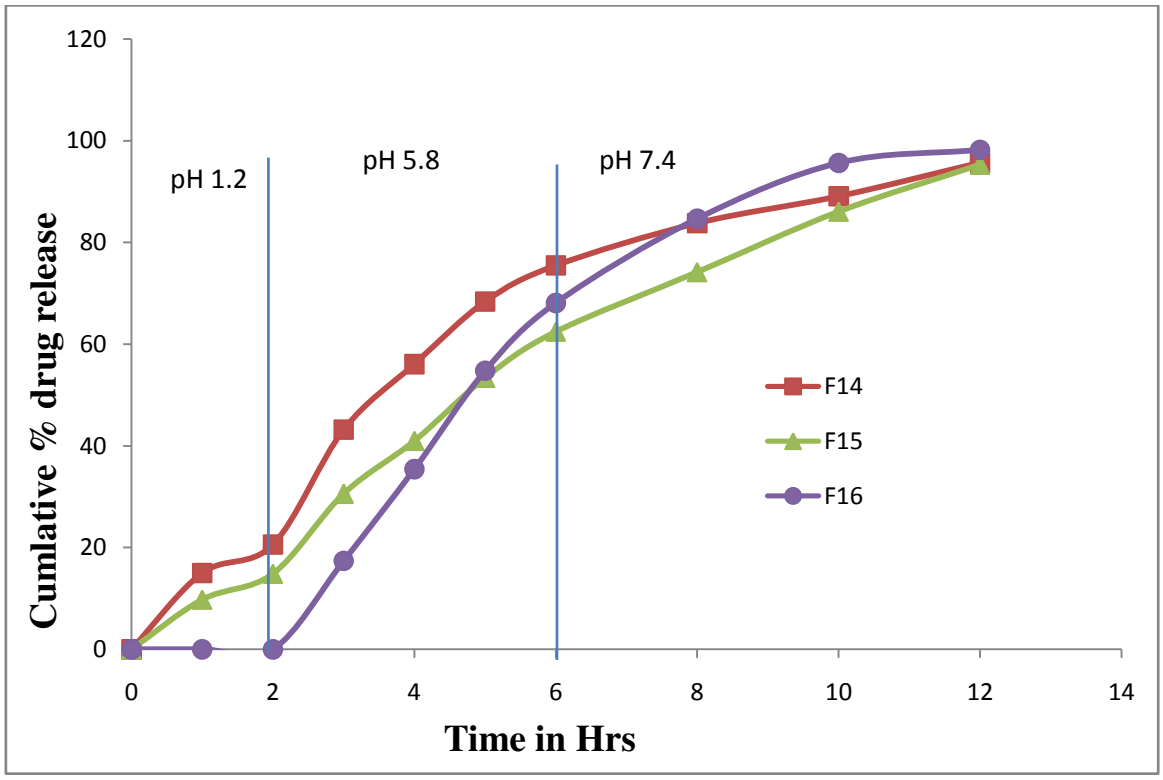

Figure: 28 Invitro- drug release study of Eudragit coated CP loaded Alginate OG blend beads

RELEASE KINETICS[82]

The results of the curve-fitting into various mathematical models like zero-order, first-order, Hixson–Crowell, Higuchi, and Korsmeyer–Peppas modelsare given in Table 13. When the respective $R^2$of Eudragit coated CP loaded alginate-OG blend beads (F-14 to F-16) were compared, it was found to follow the Higuchi model (R^2= 0.9772 to 0.986) over 12 h of in vitro drug release. In addition, Korsmeyer–Peppas model (R^2= 0.9649 to 0.9792) and was found closer to the best-fit. The value of release exponent (n) determined from in vitro CP release data of various Eudragit coated CP-loaded alginate-OG blend beads ranged from 0.77 to 1.231, indicating the super case-II transport mechanism controlled by swelling and relaxation of Eudragit coated CP loaded alginate-OG blend matrix. Results of release studies indicate that Eudragit S100 coated hydrogel beads offer a high degree of protection from premature drug release in simulated upper GIT conditions. A well coat of Eudragit S100 delivers almost intact beads to colon, an environment rich in bacterial enzymes that degrade the polysaccharides and allow drug release to occur at desired site and can be a potential system for delivery of Capecitabine in cases of colon cancer.

Table: 13 Model fitting of in-vitro release studies of Eudragit coated CP loaded Alginate OG blend beads

MODELS		**FORMULATION CODES**		
		F14	**F15**	**F16**
Zero order	R^2	0.9648	0.9644	0.9746
First order	R^2	0.8139	0.9413	0.8671
Hixson–Crowell	R^2	0.8976	0.8728	0.9129
Higuchi	R^2	0.986	0.9893	0.9772
Korsmeyer–Peppas	R^2	0.9758	0.9792	0.9649
	n	0.7796	0.8099	1.231

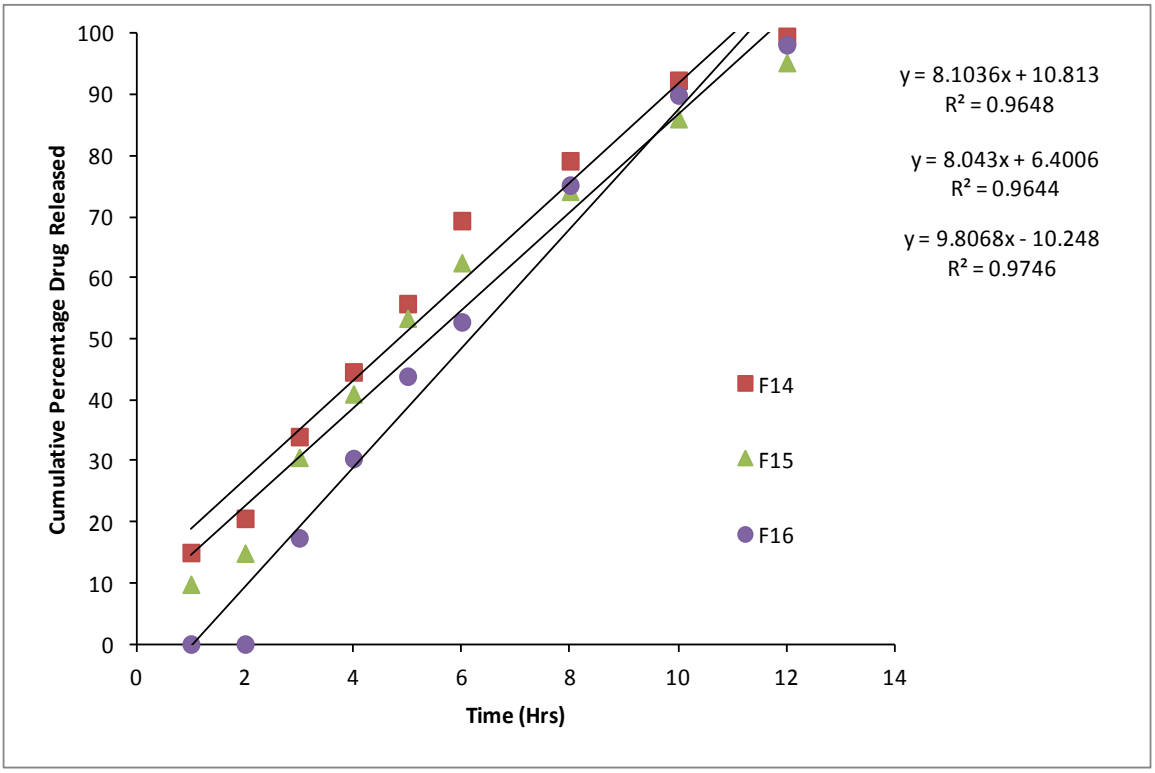

Figure: 29 Zero order kinetics of CP loaded Alginate OG blend beads

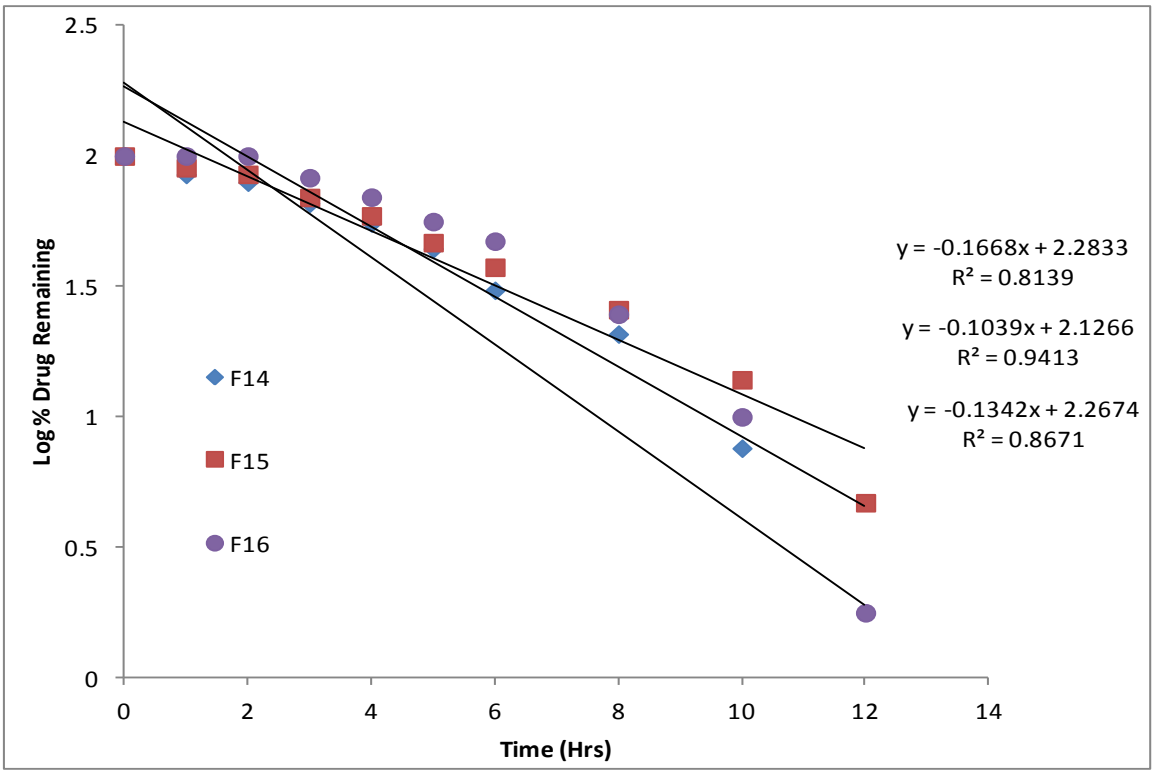

Figure: 30First order kinetics of CP loaded Alginate OG blend beads

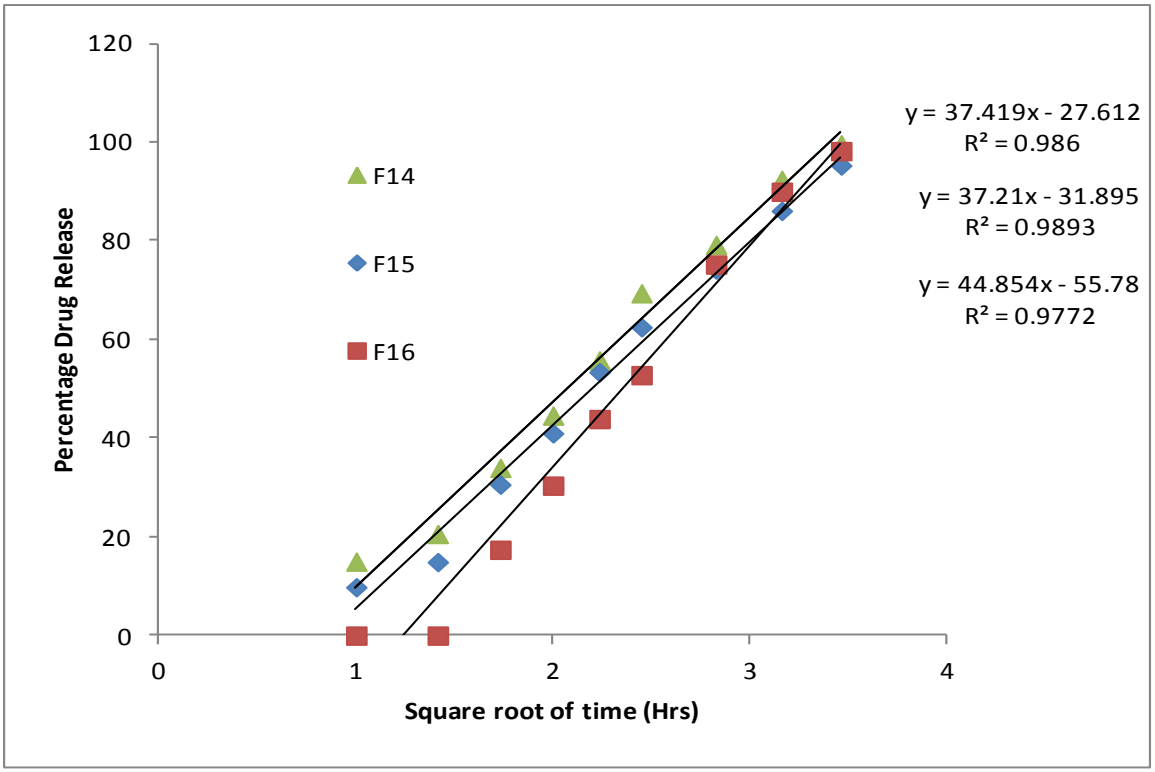

Figure: 31 Higuchi order kinetics of CP loaded Alginate OG blend beads

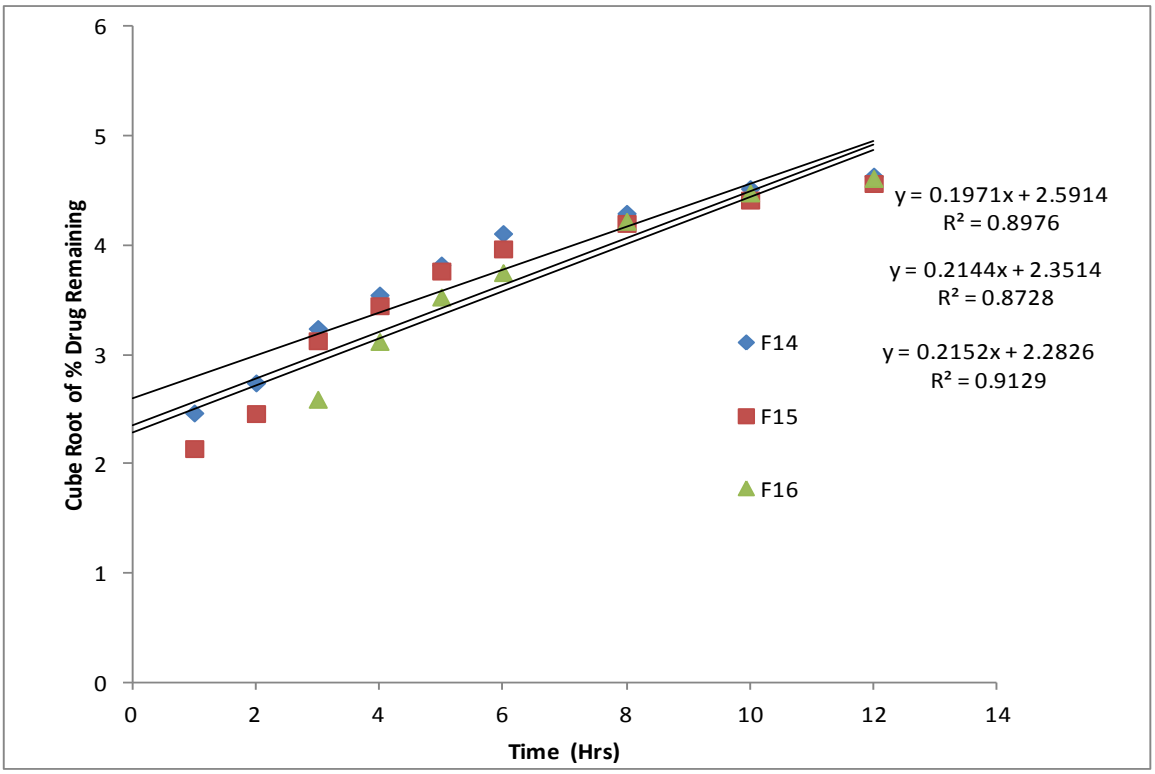

Figure: 32 Hixon- Crowel kinetics of CP loaded Alginate OG blend beads

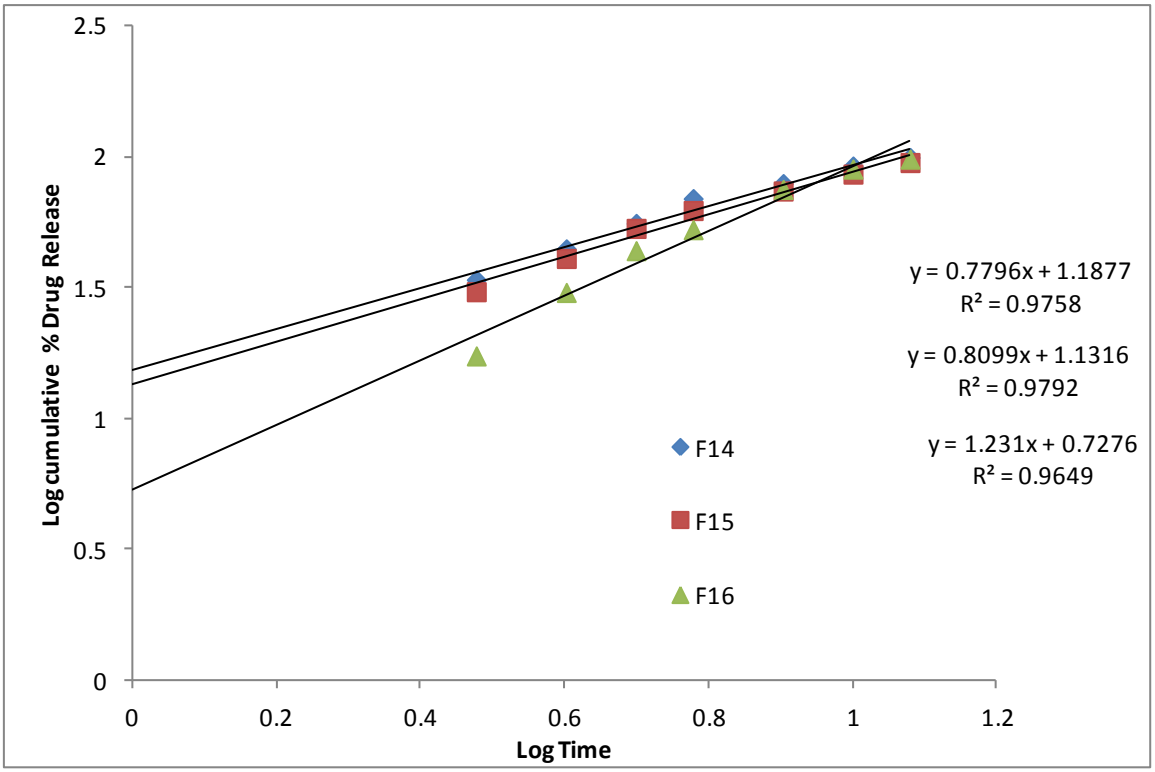

Figure: 33 Korsmeyer-Peppas model of CP loaded Alginate OG blend beads

CONCLUSION

Isolated OG was investigated as potential sustained drugrelease polymer-blends with SA in the development of controlleddrug release ionically gelled beads for oral use. Ca^{2+}-ion induced CP-loaded alginate-OG blend beads was successfully prepared by Ca^{2+}-ion induced ionic-gelation cross-linking method using $CaCl_2$as cross-linker in an aqueous environment. Effects of polymer-blend ratio and cross-linker concentration on drug loading (DL) and cumulative drug release at 12 h (R12h) wereoptimized by factorial design. The DL of these alginate-OGbeads was found increasing with the decreasing of polymer-blendratio and increasing $CaCl_2$concentration. However, an increase inR12hvalues with the increasing of polymer-blend ratio and $CaCl_2$concentration was observed. Based on the numerical optimization, optimized beads were prepared using polymers concentration (i.e., SA and OG) = 4% and $CaCl_2$ concentration = 10% w/v. Theseoptimized beads were of excellent combination of high drug loading (67.27 ± 3.58%) and suitable controlled drugrelease pattern over a prolonged period of 12h (97.73 ± 2.83%),which could possibly be advantageous in terms of advanced patientcompliance with reduced dosing interval.These Ca^{2+}-ions induced CP-loaded alginate-OG blend beads also exhibited pH-dependent swelling, which could be advantageous for intestinal drug delivery. It was found that the drug release was shown in acidic pH to retard the release in acidic pH the prepared OG beads were coated with Eudragit S-100. Coated calcium alginate beads leads to prevent release in stomach pH and upper intestinal pH and rapid release of certain amount of drug on lower intestinal pH. The in vitro drug releasefrom these Eudragit coated OG-alginate beads was followed controlled-release (Higuchi model) pattern with super case-II transport mechanism. The result suggested that novel system might have potential carrier for colon targeted delivery of therapeutic drugs.

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