DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF CAPECITABINE LOADED CHITOSAN-TRIPOLYPHOSPHATE NANOPARTICLES

Thesis submitted in partial fulfilment of the requirements for the degree of MASTER OF PHARMACY, Faculty of Engineering and Technology

By

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This is to certify that Miss Debarati Ghosh, B.Pharm, Reg. No. 129108 of 2014-2015, bearing Exam roll no. M4PHA1610, has completed her research work on the subject entitled 'DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF CAPECITABINE LOADED CHITOSAN-TRIPOLYPHOSPHATE NANOPARTICLES' under my supervision and guidance in the Department of Pharmaceutical Technology of this university. She has incorporated her findings into this thesis of the same title; being submitted by her, in partial fulfilment of the requirements for the degree of Master of Pharmacy of Jadavpur University. She has carried out this research work independently and with proper care and attention to my entire satisfaction.

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of her Master of Pharmacy studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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THESIS TITLE: "**DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF CAPECITABINE LOADED CHITOSAN-TRIPOLYPHOSPHATE NANOPARTICLES"**

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I accolade my highest respect to my parents, for their blessings, morale support, and constant encouragement and for giving me a chance to realize my passion for research, without whom it would not have been possible for me, to complete the project work in due time.

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PREFACE

This dissertation is presented for the partial fulfilment of the degree of Master of Pharmacy. The scope of the present work is development of chitosan-tripolyphosphate nanoparticles followed by its characterization and in-vitro evaluation, using Capecitabine as the model drug. Capecitabine is an anti-cancer drug used mainly for the treatment of breast cancer, colon cancer and colorectal cancer. Capecitabine tablets are commercially available but Capecitabine nanoparticles still have to go a long way to achieve the same status. Nanoparticles have many advantages over conventional dosage forms, like controlled drug delivery over an extended period of time, reduced usage of drug during production but achieving more therapeutics efficacy because of direct molecular interaction leading to reduction in dose, reduction in frequency of administration, reduction in first pass metabolism, improvement in bioavailability and thus alleviating painful traditional chemotherapy. Owing to all these advantages, extensive research work is being carried out worldwide to formulate anti-cancer drugs in the form of nanoparticles and to make it suitable for commercial usage.

In the present study Capecitabine loaded chitosan-tripolyphosphate nanoparticles were prepared by ionotropic-gelation method. After preparation, optimization was carried out by varying the concentration of chitosan. Characterization and in-vitro dissolution study were carried out for Capecitabine loaded nanoparticles and the data obtained from in-vitro study was analyzed to understand the mechanism of drug release from the nanoparticles.

This thesis is divided into various chapters, describing fundamentals, reviews of literature, aims and objectives, materials and methodologies, tables and graphs, results and discussions, conclusion, future prospects of nanotechnology and reference. Chapter 1 deals with the

introduction describing the emergence of nanotechnology along with its merits and limitations, fundamentals of nanoparticles, different methods of preparing nanoparticles, characterization, evaluation, applications and products in pipeline. Chapter 2 includes review of literature. Chapter 3 highlights the aims and objectives of the thesis work. Chapter 4 describes the materials and methodologies used. Chapter 5 includes tables and graphs. Chapter 6 is the results and discussions section which discusses the outcomes of the results obtained in due course of the experiment. Chapter 7 deals with the conclusion. Chapter 8 gives an outline of the future scope of nanoparticles, not only in the field of medical science or pharmaceutical technology but also in other fields of science. Chapter 9 contains the list of references used to successfully complete the thesis work.

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CHAPTER 1:
INTRODUCTION

1. Introduction

1.1. Basic considerations:

Nanotechnology and nanoscience have indeed taken centre stage in the arena of academic science and they are making significant headway into numerous industrial sectors. It has changed the way we describe an object and its properties. Basic nanoscience research has been in existence for over 20 years. The chemical, physical and mechanical properties of the bulk materials are different at the nanometre range. A 10nm particle has different properties when compared to the bulk of the same material. It permits a new way to vary and control the properties of materials. In fact, one needs only to change the size of particles rather than their composition to vary the properties. This is a revolution in science called Nano Revolution [1]. The Nano Revolution taking place is attributed not only to the recognition of its potential value but also to the availability of the tools that allow scientists to explore the world at the nanoscale level and transform their discoveries into products and services with practical benefits to everyday life. Nanotechnology promises to have a revolutionary impact on the way things are designed and manufactured.

A nanometre is used to measure things that are very small. Atoms and molecules, the smallest pieces of everything around us, are measured in nanometres. Only powerful microscopes like scanning electron microscope (SEM), field emission scanning electron microscopes (FESEM) and transmission electron microscope (TEM) can detect particles of nanometre scale. While some say nanotechnology deals with structures between 1nm to 100nm, some other say that nanotechnology is the branch of technology that deals with dimensions and tolerances less than 100 nm, especially the manipulation of individual atoms and molecules. According to the Royal Society and the Royal Academy of Engineering, UK, nanotechnology encompasses the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometre scale and nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale. Nanoscience is concerned with understanding the properties of the nanoscale materials and nanotechnology deals with exploiting these properties to create new devices and systems with novel properties and functions due to their size. Nanoparticles range from 100nm down to the size of atoms i.e. approx. 0.2 nm because at this scale, the properties of materials can be very different from those at a larger scale

[2]. It is this difference in the property, which is taken into account to widen our understanding of the nanoworld. The term nanotechnology encompasses a wide range of tools, techniques & potential applications, therefore the term 'nanotechnologies' is more appropriately used.

An understanding of the physics & chemistry of matter and processes at the nanoscale is relevant to all scientific disciplines. Indeed it could be argued that evolutionary developments in each of these fields towards investigating matter at increasingly small size scales has now come to be known as 'nanotechnology'.

To understand about the size range of materials to be considered 'nano', it is important to know about the definitions of the 'nano' sized materials.

- **Nanomaterials:**

a) Materials whose dimensions are in nanometres.

b) Materials with at least one dimension below 1µm but greater than 1nm are nanomaterials.

c) Nanomaterials are sometimes called nanopowders, when not compressed and have gain sizes of the order of 1-100nm in at least one coordinate.

- **Nanostructures:**

Nanostructures are those structures with at least two dimensions below 100nm. An extended definition includes structures with at least one dimension below 100 nm and a second dimension below 1µm.

1.1.1. Merits of nanomaterials / nanostructures:

Since nanomaterials and nanostructures have a little difference between them, both terms may be used synonymously throughout the thesis.

1) Nanoscale objects are more advantageous over microparticles because, microscale objects have essentially the same properties as the bulk material, whereas nanoscale objects have entirely new and unique properties than what is observed in their bulk nature. The increase in surface-area-to-volume ratio, in the case of nanoscale objects have altered the mechanical, thermal and catalytic properties of materials. This change in properties of materials when its

size is reduced to nanorange is because of the fact that matter at the nanoscale no longer follows Newtonian physics but rather it follows quantum physics.

2) Quantum mechanics predicts all sub-atomic phenomena in terms of probabilities. The mass of a nanomaterial and the gravitational forces are negligible; instead electromagnetic forces are dominant in determining the behavior of atoms and molecules.
3) In a nanomaterial, electrons are confined in a small space rather than the space of bulk material, resulting in their confinement. It is referred as quantum confinement. This is the main reason for many optical properties of the nanomaterials where the quanta of light or the electromagnetic waves interact with the confined electrons of the nanomaterial.
4) The size at which the property of a material changes entirely from the bulk of it, the material is then called to be in its nanoscale range. Usually it occurs at a size below 100nm.

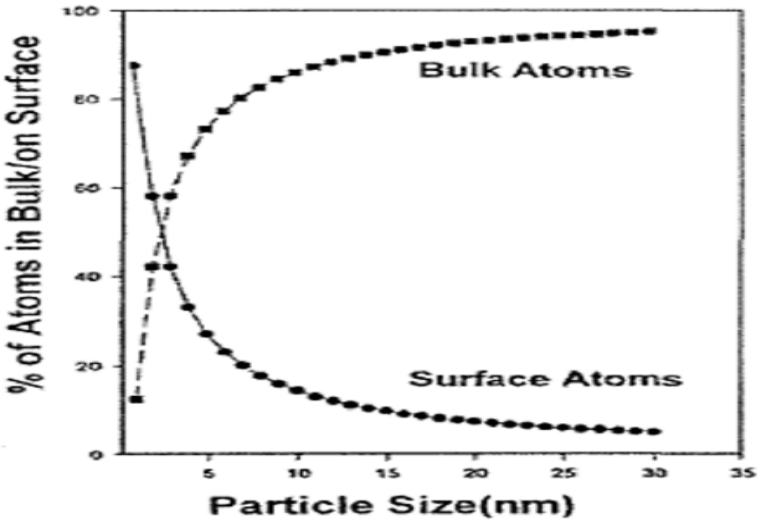


Fig1.1.The proportion of surface atoms as a function of bulk atoms.

Since the surface area to volume ratio is high in case of nanoparticles, the release of the drug is fast in case of 'drug carrier' nanoparticles like nanospheres, nanocapsules, solid-lipid nanoparticles etc. If the size is reduced, out of the total atoms or molecules that make up the nanomaterial, most of the atoms or molecules are the surface atoms or molecules. Drugs associated with these surface atoms or molecules will exhibit very fast release. Hence, lesser the size range, faster will be the drug release.

5) Due to the increased surface area, all nanomaterials possess a huge surface free energy and thus are thermodynamically unstable or metastable. One of the biggest challenges in

fabrication & processing of nanomaterials is to overcome the surface energy and to control their size for a specific property. In order to produce and stabilize the growth of nanomaterials, it is essential to have good understanding of the surface energy and surface chemistry of solids.

6) Atoms or molecules at the interface have enhanced reactivity and a greater tendency to agglomerate, so that, surface atoms and molecules are unstable and they have high surface free energy. Hence, for a nanoparticle, the surface energy is higher as compared to that of microparticles; hence the tendency to react is also high in case of nanoparticles than in case of microparticles.
7) All the properties of solids and liquids are due to the interaction of atoms and molecules. If the percentage of surface atoms changes, the properties of condensed matter correspondingly varies. If a particular property is a consequence of the interaction of a large number of molecules or atoms, it is proper to consider that the surface of this material consists of more than one layer of atoms. It is possible that for this property, three layers are to be considered to constitute the surface. As the particle size decreases below 200nm, the influence of surface atoms becomes significant and this will lead to change in that particular property. The dimension, at which significant changes in physical properties appear, is called as the characteristic length. This dimension is usually in the range of 10-100 nm. Example-change in optical property of Silicon: When a large amount of energy is absorbed by the Silicon atoms, it comes to its original state by emitting heat rather than optical radiation. However, in case of porous Silicon, light is emitted when it is finely subdivided into nanometre size wires[3].
8) An increase in efficiency of catalysts is observed with the reduction in size. Finely divided Nickel or Platinum is used as catalysts which provide a site for the reactant molecules to bind, till the reaction is complete. By using finely divided catalysts, the number of sites available for reaction increases, with increase in surface area and the catalysts become more useful. Nanoscale gold particles can catalyze chemical reactions. It has been found that finely dispersed gold nanoparticles on oxide supports are catalytically very active. In many areas the catalytic activity and selectivity of dispersed gold nanoparticles exceeds those of the commonly used transition metal catalysts such as Platinum and Palladium. This is an exciting

result because metals like Platinum and Palladium are toxic and are also very rare metals, hence very expensive.

9) The nanoscale range is of paramount importance for biologists and medical students. Biological structures are within the size that researchers are now able to manipulate and control. The size of nanomaterials is similar to that of most biological molecules and structures. Therefore, nanomaterials can be used both in *in-vivo* and *in-vitro* biomedical research and applications. Medical researchers work at the microscale and nanoscale levels to develop new drug delivery methods for therapeutics and pharmaceuticals. Engineered devices at the nanoscale level are small enough to interact directly with sub-cellular compartments and to probe intracellular events.
10) There has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules.
11) In recent years, the pharmaceutical and biotechnology industries have developed more sophisticated and potent drugs. Many of these agents are proteins on DNA; the therapeutic window (i.e. the range of concentrations that bracket the effective and toxic regimes for the drug) for these drugs is often narrow; and the toxicity is observed for concentration spikes, which renders traditional methods of drug delivery ineffective[4]. In addition, conventional oral doses of these agents are frequently useless, because the drugs are destroyed during intestinal transit or are poorly absorbed. Hence novel methods of drug delivery have been synthesized among which nanoparticles are extensively researched because it is predicted to stand out as one of the commonly used mode of drug delivery in the near future.

1.1.2. Limitations of nanomaterials/ nanostructures:

According to the fundamental concepts, a system of high surface free energy will strive to attain a state of lower energy by whatever means possible. Nanomaterials are inherently unstable because atoms or molecules at the interface have enhanced reactivity owing to its high surface energy and thus have a greater tendency to agglomerate. Hence, nanoparticles tend to precipitate because of its unstable nature.

1.1.3. Unique properties of nanomaterials / nanostructures:

1) In a nanomaterial, electrons are confined within a small space. This phenomenon is referred to as quantum confinement, for which many optical properties of the nanomaterial varies as compared to that of its bulk counterpart. In nanomaterials, the quanta of light or electromagnetic waves interact with the confined electrons of the nanomaterial.
2) The origin of quantum confinement in so called zero-dimensional nanocrystals is understood to arise from the spatial confinement of electrons within the crystallite boundary. The properties of a material depend on the type of motion its electrons can execute, which in turn depends on the space available to them. As the size of the material decreases, the movement of electrons becomes restricted. It leads to a larger spacing in between the energy levels and more energy is required to excite the electrons. The quantum confinement of electrons hence can control the properties of nanomaterials.
3) The properties of materials can be different at the nanoscale for two main reasons. First, nanomaterials have a relatively larger surface area when compared to the same mass of material produced in a larger form. This can make materials more chemically reactive (in some cases materials that are inert in their larger form are reactive when produced in their nanoscale form),and affect their strength or electrical properties. Second, quantum effects can begin to dominate the behavior of matter at the nanoscale - particularly at the lower end - affecting the optical, electrical and magnetic behaviour of materials. Materials can be produced that are nanoscale in one dimension (nanowires and nanotubes) or in all three dimensions (nanoparticles).
4) As the percentage of surface atoms change, the properties of condensed matter correspondingly vary.

1.1.4. Types of nanomaterials / nanostructures:

1) Quantum well - Nanobricks, nanobelts, nanofilms, nanosheets.
2) Quantum wires - Nanorods, nanofibres, nanotubes.
3) Quantum dots - Nanoparticles, nanocrystals.

1) Quantum well

a) The dimension of one is reduced to nano range while the other two dimensions remain large.

b) A quantum well is simply a thin film of macroscopic width and length, large enough to be visible to naked eye but only few nanometers in thickness. Thus it is said to be confined in one dimension, or confined within two dimensions.

c) Quantum wells are materials that have one dimension in the nanoscale range (are extended in the other two dimensions) are layers, such as thin films or surface coatings. Ex: Some of the features on computer chips come in this category.

2) Quantum wires

a) Two dimensions when reduced and one remains large, the resulting structure is called as quantum wire.

b) Quantum wires which are confined in two dimensions can be thought of as nanometre-sized cylinders that can measure several microns in length. These are structures confined within one dimension.

c) Materials that are nanoscale in two dimensions (and extended in one dimension) include nanowires and nanotubes.

3) Quantum dots

a) Quantum dot is a zero-dimensional nanometre sized semiconducting sphere, confined in all three dimensions.

b) Materials that are nanoscale in three dimensions are particles, for example- precipitates colloids and quantum dots (tiny particles of semiconductor materials). Nanocrystalline materials, made up of nanometre sized grains, also fall into this category.

Table 1.1.Examples of reduced dimensionality systems.

2D Structures	1D Structures	0D Structures
Nanofilms	Nanorods	Nanoparticles
Quantum wells	Nanowires	Quantum dots
Grain boundary films	Carbon nanotubes	Fullerenes

Types of Nanoparticles:

One can distinguish two types of nanoparticles –

I. Nanospheres
II. Nanocapsules.

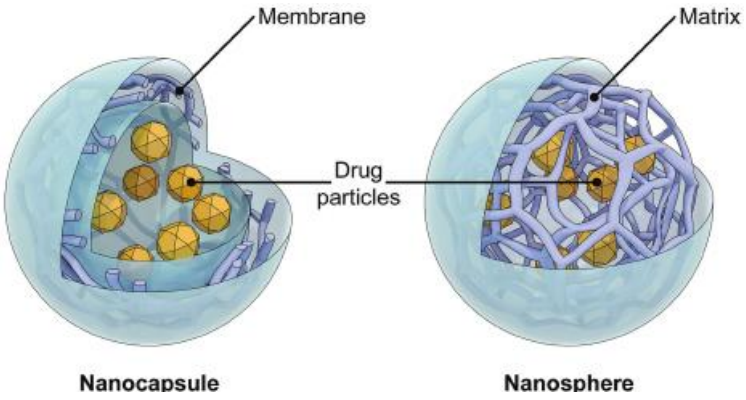


Fig 1.2. Types of Nanoparticles.

The term PNP is a collective term for any type of polymeric nanoparticles, but specifically for nanospheres and nanocapsules.

I. Nanospheres:

a) Matrix systems, where the whole particle consists of a continuous polymer network

b) Nanospheres are easier to prepare than nanocapsules and were developed much before nanocapsules.

c) The drug is physically and uniformly dispersed within the matrix system.

d) Emulsion polymerization, Nanoprecipitation, Salting out, Ionotropic gelation methods give rise to nanospheres.

e) Doxorubicin, an anticancer agent, a peptide growth hormone and several antibiotics are prepared in the form of nanospheres.

f) Methylidene malonate is a biodegradable polymer used to make nanospheres. Poly (alkyl-cyanoacrylate) is also used.

g) Oral administration, intravenous administration, parenteral (IV, IM, SC), inhalation (for sprays and aerosols) and topical gels are the most commonly used routes of nanospheres administration.

h) Nanospheres are matrix particles, i.e. particles whose entire mass is solid and molecules may be adsorbed at the sphere surface or encapsulated within the particle. In general they are spherical, but "nanospheres" with a non-spherical shape are also described in the literature [5].

II. Nanocapsules:

a) Reservoir systems composed of a polymer membrane or shell, surrounding an oily or an aqueous core.

b) Nanocapsules are difficult to prepare as compared to nanospheres and were developed since 1986.

c) The drug is confined to a cavity surrounded by a unique polymer membrane.

d) Interfacial polymerization of alkylcyanoacrylates, Solvent evaporation, Nanoprecipitation, Rapid dispersion of an ethanol phase including ethanol, the oil, the monomer and the molecule to be encapsulated in an aqueous solution of surfactant, leads to the formation of nanocapsules.

e) Generally oil soluble drugs are formulated as oil-containing nanocapsules, however in case of highly water soluble molecule like insulin, can be entrapped in nanocapsules with up to 97% encapsulation yield.

f) Alkylcyanoacrylate biodegradable polymers are generally used to make nanocapsules.

g) Oral administration, intravenous administration, parental (IV, IM, SC), inhalation (for sprays and aerosols) and topical gels are the most commonly used routes of nanocapsules administration

h) Nanocapsules are vesicular systems, acting as a kind of reservoir, in which the entrapped substances are confined to a cavity consisting of a liquid core (either oil or water) surrounded by a solid material shell [6].

1.1.5. Utility of surface modification of conventional nanoparticles:

1) To minimize opsonization and to prolong the circulation of nanoparticles *in-vivo*, the surface of nanoparticles is coated with biodegradable hydrophilic polymers/surfactants such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80).

2) Surface modification is done for increasing the targeted action of nanoparticles towards specific cell types. Stella *et al.* prepared poly (alkyl-cyanoacrylate) nanoparticles showing residues of folic acid on their surface. These nanoparticles were used to target cancer cells overexpressing a membrane

receptor for folic acid. The targeting moiety, consisting of folic acid was grafted on the surface of poly (aminopoly (ethylene glycol) cyano-co-hexadecylcyanoarylate) nanoparticles that were obtained by Nanoprecipitation [7].

3) Nanoparticles are able to escape complement activation through surface modification.Passirani *et al*.[8] proposed to coat nanospheres with heparin. Heparin is a polysaccharide and a physiological inhibitor of complement activation *in-vivo*.

4) Surface modifications are done to improve the bioadhesivity of nanoparticles to the particular mucosae.

1.1.6. Nanobiomaterials:

Scientists are trying to mimic nature's designs to develop biomaterials which can be used in replacement of diseased or damaged human body parts. These materials must not cause adverse biological reactions in the body. All of the materials such as metals, ceramics, polymer composites and semiconductors can be employed as biomaterials. Nanoscale materials transcend the entire spectrum of biological substances. Nanomaterials employed as biomaterials are called nanobiomaterials. Research shows that all living systems are governed by molecular behavior at the nanometer scale. The molecular building blocks of life such as proteins, nucleic acids, lipids and carbohydrates possess unique properties determined by their size, folding and patterns at the nanoscale. Specifically the organization of cells and the corresponding tissue properties are found to be highly dependent on the structure of the extra-cellular matrix [9]. The extra-cellular matrix has a complex hierarchical structure with spectral and temporal levels of organization that span several orders of magnitude. For these reasons, cells in our body are predisposed to interact with nanostructured surfaces. Indeed, there is research evidence that a biomaterial composed of nanometre-scale components is biologically preferred. Nanobiomaterials have a large number of atoms and crystal grains at their surfaces and possess a higher surface-area-to-volume ratio than conventional microscale biomaterials. These differences in surface topography alter the corresponding surface energy for protein adsorption.

1.1.7. Polymer selection:

Various polymers have been used in the formulation of nanoparticles for drug delivery research, to increase therapeutic benefits, while minimizing side effects. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers.

The selection of matrix materials is dependent on [10]

i) Size of nanoparticles required.

ii) Inherent properties of the drug e.g. aqueous solubility and stability.

iii) Surface characteristics such as charge and permeability.

iv) Degree of biodegradability, biocompatibility and toxicity.

v) Drug release profile desired.

vi) Antigenicity of the final product.

Polymers used to prepare nanoparticles**:**

- Biodegradable
  - i) Natural: Proteins, Gelatin, Albumin, Lectins, Legumin.
  - ii) Polysaccharides: Chitosan, Dextran, Alginate, Agarose, Pellulan, Allylic starch.
  - iii) Synthetic: PLA, Polylactic acid; PCL, Poly (ε-Caprolactone); PLGA, poly (lactic-co-glycolic acid); PEG, Polyethylene glycol.
- Non-biodegradable
  - i) These are synthetic polymers like Acrylamide, Polyamide and Polystyrene.

Chitosan:

1) Chitosan polymers possess complex structure that may contain a wide range of nanocrystalline material and are held together by covalent and weaker secondary forces.

2) Chitosan has good bioadhesive properties and absorption / permeation enhancing capacity.

3) Chitosan is non-toxic and biodegradable in nature.

4) Chitosan is hydrophilic and hence prevents opsonization.

5) Chitosan is also biocompatible.

6) Low molecular weight (LMW) chitosan, compared to High molecular weight (HMW) chitosan shows better solubility, biocompatibility, bioactivity, biodegradability and less toxicity[11-13].

7) Chitosan has the ability to control the release of active agents and is also soluble in aqueous acidic solution.

8) It is a linear polyamine containing a number of free amine groups that are readily available for cross-linking, its cationic nature allows for ionic cross-linking with multivalent anions.

9) Chitosan has a mucoadhesive character, which increases the residual time of the drug, at the site of absorption. Ionic interactions between positively charged amino groups in chitosan and the negatively charged mucus gel layer make it mucoadhesive. The favorable properties like biocompatibility, biodegradability, pH sensitiveness, mucoadhesiveness, etc. has enabled these polymers to become the choice of the pharmacologists as oral delivery matrices for proteins.

10) Chitosan has an ability to facilitate paracellular transport of hydrophilic drugs across mucosa. Chitosan forms non-covalent bonds between itself and the mucus glycoprotein, thus though chitosan is hydrophilic, it can improve the permeation of hydrophilic drugs through biological barriers.

Gelatin:

Gelatin is an irreversibly hydrolyzed form of collagen, wherein the hydrolysis results in the reduction of protein fibrils into smaller peptides, which will have broad molecular weight ranges associated with physical and chemical methods of denaturation, based on the process of hydrolysis.

Lectins:

Lectins are carbohydrate-binding proteins, macromolecules that are highly specific for sugar moieties. Most lectins do not possess enzymatic activity. Lectins occur ubiquitously in nature. They may bind to a soluble carbohydrate or to a carbohydrate moiety that is a part of a glycoprotein.

Legumin:

Legumin, or vegetable casein, is a protein substance analogous to the casein of milk, obtained from beans, peas, lentils and other leguminous seeds. It is a member of the goblin group. Legumin is soluble in water and is soluble in very weak acids and alkalies; and it is not coagulated by heat.

1.1.8. Nanofabrication:

There are two generalized processes for nanofabrication:

1) Top-down methods (physical/hard methods)

2) Bottom-up methods (chemical/soft methods).

1) Top-down methods

- Involves continuous breaking up of the bulk matter to atomic or molecular dimension.
- Begins with a pattern generated on a larger scale, then reduced to nanoscale.
- By nature, are not cheap and quick to manufacture.
- Slow and not suitable for large scale production.

A top down synthesis method implies that the nanostructures are synthesized by etching out crystals planes (removing crystal planes) which are already present on the substrate. A top-down approach can thus be viewed as an approach where the building blocks are removed from the substrate to form the nanostructure.

2) Bottom-up methods

- Starts with atoms or molecules and builds up to nanostructures.
- Fabrication is much less expensive.
- Easy but time consuming.

A bottom up synthesis method implies that the nanostructures are synthesized onto the substrate by stacking atoms onto each other, which gives rise to crystal planes. Crystal planes further stack onto each other, resulting in the synthesis of nanostructures. A bottom-up approach can thus be viewed as a synthesis approach where the building blocks are added onto the substrate to form nanostructures.

Chemical synthesis is a bottom-up process. The advantages of bottom-up processes over top-down processes are that nanostructures with fewer defects are obtained. More homogeneous chemical composition and better long and short range ordering are observed in case of bottom-up methods. This is because the bottom-up approach is driven mainly by the reduction of Gibbs free

energy, so that the nanostructures produced are in a state closer to a thermodynamic equilibrium state.

Preparation of polymeric nanoparticles is a bottom-up approach and are generally of two types

A. Dispersion of preformed polymers B. Polymerization of monomers

The choice of preparation method is made on the basis of a number of factors such as the type of polymeric system, area of application, size requirement, etc. For instance, a polymeric system that is developed for an application in the pharmaceutical or environmental fields should be completely free from additives or reactants such as surfactants or traces of organic solvents. In order to achieve the properties of interest, the mode of preparation plays a vital role.

A. Dispersion of preformed polymers

1. Solvent Evaporation:

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration[14]. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed [15].

Polymers used: PEG, poly (ethyleneglycol); PLLA, poly (l-lactic acid); PLGA, poly (d, l-lactic acid-co-glycolic acid);POP, poly (organophosphazene).

2. Salting-out:

Allemann *et al*. [4] carried out a typical process where an electrolyte-saturated, or a nonelectrolyte-saturated aqueous solution containing PVA as a viscosity – increasing agent and stabilizer, was added to an acetone solution of the polymer under constant stirring. The saturated aqueous solution prevented the acetone from mixing with the water by a salting-out process. After the preparation of an oil-in-water emulsion, water was added in a sufficient amount to

allow the complete diffusion of acetone into the aqueous phase, resulting in the formation of nanospheres.

Polymers used: EUDRAGIT L100-55; PEO, poly (ethylene oxide); PMA, poly (α, β,-l-malic acid); PTMC, poly (trimethylene carbonate).

3. Nanoprecipitation (Solvent displacement method):

The basic principle of this technique is based on the interfacial deposition of a polymer after displacement of a semi-polar solvent, miscible with water, from a lipophilic solution. Nanoprecipitation systems consist of three basic components - the polymer (synthetic, semi-synthetic or natural), the polymer solvent and the non-solvent of the polymer. Organic solvent (i.e., ethanol, acetone, hexane, methylene chloride, dioxane, dichloromethane) which is miscible in water and easy to remove by evaporation is chosen as polymer solvent. Due to this reason acetone is the most frequently employed polymer solvent used in this method [15-16].

This method involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of surfactants [17-20]. The polymer, generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as the surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension [21]. The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared.

Polymers used: PLGA, poly (d, l-lactic acid-co-glycolic acid); PHB, poly (hydroxyl butyrate); PCL, poly (ε-caprolactum); Allylic starch, Dextran ester.

4. Ionotropic-gelation method

Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionotropic gelation [22-23].This method has been developed with alginate and chitosan that form

highly water swollen gels. The gelling agents for these two natural polysaccharides are respectively calcium and tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Ionic gelation technique is based on the ionic interactions between the positively charged primary amino groups of chitosan and the negatively charged groups of polyanion; such as sodium tripolyphosphate (STPP), which is the most extensively used ion cross-linking agent due to its non-toxic and multivalent properties[24].

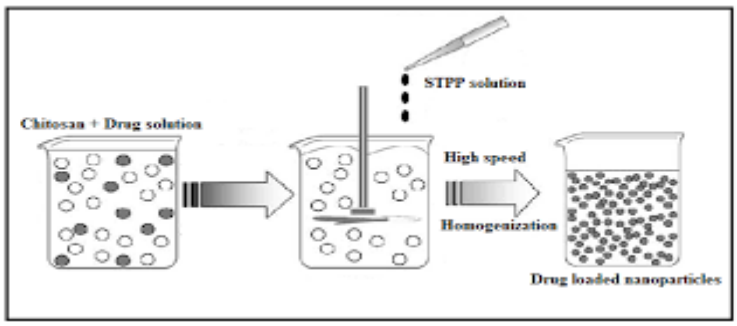


Fig 1.3. Schematic representation of Ionotropic-gelation method.

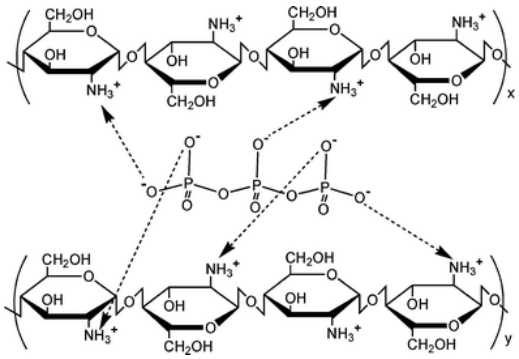


Fig 1.4. Ionic cross linking between chitosan and TPP.

Polymers used: Biodegradable hydrophilic polymers like chitosan, gelatin and sodium alginate.

B.Polymerization of monomers

1. Emulsion polymerization:

Emulsion polymerization is the most common method used for the production of a wide range of specialty polymers. In emulsion polymerization the monomer itself, if liquid is dispersed under

agitation in a continuous phase in which it is non-miscible. The polymerization is usually initiated by the reaction of the initiators with the monomer molecules that are dissolved in the continuous phase of the emulsion. The polymerization continues by further addition of monomer molecules that diffuse towards the growing polymer chain through the continuous phase. The growing polymer chain remains soluble until it reaches a certain molecular weight for which it becomes insoluble. Therefore, phase separation occurs leading to the nucleation of the polymer particles and the formation of the tyndall scattering effect. Further growth of the nucleated particles occurs according to a mechanism that depends on the stability conditions of the whole system. This includes capture of new growing polymer chains, fusion or collision between nucleated particles. Throughout the polymerization, the monomer input in the continuous phase of the emulsion takes place by diffusion from the monomer droplets, which plays the role of monomer reservoirs. When the reaction is completed, the particles formed contain a large number of polymer chains. Based on the utilization of surfactant, it can be classified as conventional and surfactant-free emulsion polymerization. Emulsion polymerization can be performed in emulsifier free systems and in both oil –in-water and water-in-oil emulsion. The poly (alkylcyanoacrylate) nanospheres, widely used as drug carriers, are prepared by emulsion polymerization according to a method initially introduced by Couvreur *et al*.[25]. The monomers (isobutylcyanoacrylate, isohexylcyanoacrylate, n-butylcyanoacrylate) are dispersed in a continuous acidified aqueous phase under magnetic agitation. The anionic polymerization of the alkylcyanoacrylate is rapidly and spontaneously initiated by the remaining $–OH^-$ ions of the acidified water and is completed within 3-4 h depending on the monomer type. The preparation is performed at low pH (~2.5) to slow down the anionic polymerization of the alkylcyanoacrylate, therefore allowing the polymer to arrange as colloidal particles.

Monomers used: MMA, methyl methacrylate; HEMA, hydroxylethyl methacrylate.

Initiator used: KPS, potassium persulfate; AIBN, 2, 2′-azobisisobutyronitrile.

Surfactant used: SDS, sodium dodecyl sulfate; PVA, poly (vinyl alcohol).

2. Interfacial polymerization:

Nanocapsules can be prepared by Interfacial polymerization of alkylcyanoacrylates [7].Oil containing nanocapsules were prepared by the rapid dispersion of an ethanol phase including ethanol, the oil, the monomer and the molecule to be encapsulated in an aqueous solution of surfactant. When the ethanol diffuses in the aqueous phase, tiny individual oil droplets form, and because of the contact with water at the oil/water interface, the polymerization of the alkylcyanoacrylate takes place on the droplet surface [28-31].This method is mainly adapted for the encapsulation of oil soluble substances. Water containing nanocapsules can be obtained by the interfacial polymerization of monomers in water-in-oil micro-emulsions. In these systems, the polymer formed locally at the water-oil interface and precipitated to produce the nanocapsules shell [32, 33].

Continuous phase: Span 80, KPS, potassium persulfate; Ethylene glycol, Tween 80, Water and Butanol.

Dispersed phase: Aniline, Acetone, Span 85, Ethanol and Toluene.

1.1.9. Importance of nanotechnology in the field of cancer treatment:

1) Nanoparticles are used for the treatment of malignant tissues, due to their ability to provide uniform heating, non-invasive delivery and multiple treatments to the tissue cells. In such cases, to destroy the cells selectively, the temperature of the tissue is increased to more than 42°C. To achieve this, dose of magnetic nanoparticles can be injected into a region of malignant tissues. Then alternating magnetic field can be applied. With sufficiently strong field and optimum frequency, the particles absorb energy and heat from surrounding tissue, affecting only infected cells. Magnetic materials inside the body generate heat due to Brownian motion and Eddy currents.

2) An anticancer drug, when given intravenously is distributed throughout the body as a function of the physicochemical properties of the molecule. A pharmacologically active concentration is reached in the tumor tissue at the expense of massive contamination of the rest of the body. For cytostatic compounds, this poor specificity raises a toxicological problem, which presents a serious obstacle to effective therapy. The use of colloidal drug carriers could represent a more rational approach to specific cancer therapy. In addition, the possibility of overcoming multidrug resistance might be

achieved by using cytostatics - loaded nanospheres. For Doxorubicin loaded nanoparticles, it was found that, there was improved efficacy of the targeted drug with reduced toxic effects.

3) Alteration of the drug distribution profile by linkage to nanospheres can reduce the toxicity of a drug because of reduced accumulation in organs where the most acute toxic effects are exerted. It was found that Doxorubicin associated with nanospheres were less toxic than free Doxorubicin.

4) The ability of tumor cells to develop simultaneous resistance to multiple lipophilic compounds represents a major problem in cancer chemotherapy. Nanospheres loaded with Doxorubicin were found to effectively introduce a cytotoxic dose of Doxorubicin into the pleiotropic resistant human cancer cell line Doxorubicin R MCF 7.

5) Nanoparticles have the advantage of targeting cancer by simply being accumulated and entrapped in tumors (passive targeting). The phenomenon is called the enhanced permeation and retention effect, caused by leaky angiogenetic vessels and poor lymphatic drainage and has been used to explain why macromolecules and nanoparticles are found at higher ratios in tumors compared to normal tissues.

6) Nanoparticles are designed to safely reach their target and specifically release their cargo at the site of the disease; this way increasing the drug's tissue bioavailability. The use of such nanoparticles as delivery vehicles ensures that their cargo exerts its effect only inside the targeted cells.

7) The compounds used in cancer chemotherapy are often highly toxic to many cell types, so targeting is crucial to minimizing collateral damage to healthy bystander cells. Efficient targeting thus significantly lowers the risk of serious side-effects, while allowing the dose required for a meaningful clinical response to be reduced.

8) The primary goals for research of nano-bio-technologies in drug delivery include:

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility, and
- Faster development of new safe medicines.

9) Nanoparticles as drug delivery systems enable unique approaches for cancer treatment. Nanoparticles are often used to deliver drugs, especially those that are highly toxic, directly to

cancer cells. More recently developed nanoparticles are demonstrating the potential sophistication of these delivery systems by incorporating multifunctional capabilities and targeting strategies, in an effort to increase the efficacy of these systems against the most difficult challenges in cancer treatment, including drug resistance and metastatic disease.

10) Together with the progression of nanoscale drug delivery systems, advances in nanoscale imaging suggest the potential for the development of multifunctional "smart" nanoparticles that may facilitate the realization of individualized and targeted cancer therapy. Almost all types of nanoparticles including polymeric nanoparticles, nanocrystals, polymeric micelles, dendrimers, and carbon nanotubes have been evaluated for their suitability as multifunctional nanoparticles that can be applied for diagnostics imaging and treatment of cancers.

11) The alliance of nanotechnology and medicine has yielded an offspring that is set to bring momentous advances in the fight against a range of diseases. Cancer nano therapeutics is rapidly progressing and being implemented to solve several limitations of conventional drug delivery systems such as nonspecific bio distribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic indices.

12) Cancer therapies are currently limited to surgery, radiation and chemotherapy. All three methods risk damage to normal tissues or incomplete eradication of cancer. Nanotechnology offers the means to aim therapies directly and selectively at cancerous cells.

1.1.10. Marketed nanoparticles for cancer therapy:

Although nanotechnology is revolutionizing the diagnosis and treatment of a number of cancers, it is only six years since the first Nano particulate drug delivery product for the treatment of breast cancer, Abraxane, was launched by Abraxis Oncology, a division of American Pharmaceutical Partners, Inc. (now Abraxis Biosciences) the initial announcement saw the company′s share prices rise by 50% and required the Food and Drug Administration (FDA) to create a new class of therapeutic products. But this was only the opening shot in the war against cancer [34].

There are many companies that are emerging in the field of using nanoparticles, for drug delivery systems. Some of the companies are: Access Pharmaceuticals - an emerging biopharmaceutical company specializing in products for cancer and supportive care. The company's lead

development candidate for the treatment of ovarian cancer is ProLindac™, a nanopolymer DACH platinum prodrug [34].

Table 1.2. Products in pipeline.

Company	Technology	Active Pharmaceutical Ingredient	Route of Administration
Novavax, USA	Micellar nanoparticles	Testosterone	Subcutaneous
Bioalliance, France	Poly Hexyl cyanoacrylate nanoparticles	Doxorubicin	Intravenous
American Bioscience, USA	Albumin-Drug nanoparticles	Paclitaxel	Intravenous
Wyeth Pharmaceutical, USA	Drug Nanoparticles	Rapamycin	Oral
BioSante, USA	Calcium phosphate nanoparticles	Insulin	Oral

1.1.11. Uses of nanoparticles:

Uses of Nanoparticles for Oral Delivery of Peptides and Proteins:

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration [35].

The surface area of human mucosa extends to 200 times that of skin [36]. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g., (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin;(b) proteolytic enzymes at the brush border membrane(endopeptidases); (c) bacterial gut flora; and (d) mucus layer and epithelial cell lining itself [37]. The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate matter from the environment. One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract.

Uses of Nanoparticles in diagnosing cancer:

For diagnosing the cancer types and to detect the disease, different types of diagnostic methods are used, which include MRI, tomography, imaging and CAT scan. Microbeam radiation therapy (MRT) can be enhanced by the prior administration of gadolinium-based nanoparticles to the patient. The nanoparticles also improve contrast in magnetic resonance imaging (MRI) permitting localization of the tumor.

Computed Tomography-A CT scan may be used to make sure a procedure is done correctly. For example, the doctor may use CT to guide a needle during a tissue biopsy, or to guide the proper placement of a needle to drain an abscess. Nanoparticle types like manganese oxide particles are used as contrasting agent in MRI scan thus, accounting to its wide application. Apart from MRI scanning, the nanoparticles, especially gold, are used as optical resonance factor for tomography. Gold nanoparticles over the recent years have been used for targeted drug delivery to cancerous cells. A preferred form of delivery for gold nanoparticles is liposome mediated, as liposomes increase stability via encapsulation and moreover they are non-toxic and biocompatible.

Diagnostic nanosensors allow for early detection of various diseases, like cancer, at the very onset of the symptoms, before the disease is perceived by the patient. Production of a cocktail of different colored quantum dots helps physicians spot early indication of cancer or identify different types of tumours.Nanoparticles are being developed that recognize proteins produced only by cancer cells. Early detection means a higher chance of a successful treatment [38].

Uses of Nanoparticles in drug delivery:

Nanotechnology offers great visions of improved and personalized treatment of critical illness. The hope is that personalized medicines will make it possible to develop and administer the appropriate drug, at the appropriate dose, at the appropriate time to the appropriate patient. The benefits of this approach are accuracy, efficacy, safety and speed.

Mersana Therapeutics, Inc. is a privately held, venture-backed company that utilizes its proprietary nanotechnology platform to transform existing and experimental anti-cancer agents into new, patentable drugs with superior pharmaceutical properties. Mersana's key component of this platform is a 'stealth' material derived from dextran called Fleximer. Fleximer is a biodegradable, hydrophilic and multivalent polymer that can be chemically linked to small molecules and biologics to enhance their pharmacokinetics and safety [39].

Uses of Nanoparticles in Diagnostics:

A highly promising use of nanotechnology in diagnosis of diseases is one of its most important applications in medicines. The key principles are the specific recognition of a molecule linked to a disease state and its detection. Nanotechnology offers the prospect of very sensitive recognition and very quick detection. Quick blood tests are being developed using nanoshells coupled to molecules. Changes to the nanoshells (optical properties) when they bind to the target can easily be detected.

Uses of Nanoparticles in Biotechnology:

Nanobiotechnolgy (also called as nanobiology) evolves to deliver bio-macromolecules and molecular machines that are similar in nature. Nanobiotechnolgy encompasses research and technology developments which tend at the atomic, molecular and macromolecular levels, at scales typically below 100 nm. It is best described as helping modern medicine progress from treating symptoms to generating cures and regenerating biological tissues. An example of current nanobiotechnological research involves nanospheres coated with fluorescent polymers. Researchers are seeking to design polymers whose fluorescence is quenched when they encounter specific molecules. Different polymers would detect different metabolites. The polymer-coated spheres could become part of new biological assays, and the technology might someday lead to particles which could be introduced into the human body to track down metabolites associated with tumors and other health problems[40].

Uses of nanoparticles in parenteral:

The most commonly used route of nanoparticle administration is the parenteral route, owing to the fact that the effects of the medication are much rapid and that it can be administered directly to the site. Carcinoma and sarcoma classes of cancer are treated by using the parenteral routes of action, more specifically the intravenous route. Lipid nanoparticles mainly solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC) are used in parenteral delivery of pharmaceutical actives. The attempts to incorporate anticancer agents, imaging agents, antiparasitics, antiarthritics, genes for transfection, agents for liver, cardiovascular and central nervous system targeting have been extensively researched and are in the embryonic stage, promising an enormous growth in the near future. Stealth® nanoparticles have the ability to extravasate across the endothelium that becomes permeable due to the presence of solid tumors and are often administered intravenously [41].

1.1.12. Characterization of Nanoparticles:

1) Particle size

- The particle size and the particle size distribution of polymeric nanoparticles are of great importance because they determine their key properties such as viscosity, surface area and packing density.
- The size as well as the size distribution are important parameters to be determined to achieve safe intravenous administration.
- Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the *in-vivo* distribution, biological fate, toxicity and the targeting ability of nanoparticle systems.
- Particle size and distribution can also influence the drug loading, drug release and stability of nanoparticles.
- Drug release is affected by particle size. Smaller the particles, greater is the surface area for interaction with the surrounding environment, leading to a faster release of the drug from its dosage form.
- For measuring the particle size and size distribution of nanoparticles, a technique called quasi-elastic light scattering or photon correlation spectroscopy or dynamic light scattering is used.

This method is based on the evaluation of the translational diffusion coefficient, D, characterizing the Brownian motion of the nanoparticles. The nanoparticle hydrodynamic diameter, d_H is then deduced from this parameter using the Stokes - Einstein equation :

$$D = \frac{1.38 \times 10^{-12} \times T}{3\pi\eta d_H} m^2 s^{-1}$$

Where, D = Translational diffusion coefficient

T = Absolute temperature (Kelvin)

η= Fluid viscosity

d_H = Hydrodynamic diameter.

Photon correlation spectroscopy analyses the constantly changing patterns of laser light scattered or diffracted by particles in Brownian motion and monitors the rate of change of scattered light during diffusion.

Fig 1.5.Malvern Zeta-sizer instrument for dynamic light scattering.

2) Polydispersity index

- The Polydispersity index (PDI) helps in determining the degree of homogenicity of the nanoparticle dispersion. It is measured within a range of 0 to 1. A lower value of PDI indicates that the system is homogeneous, with a uniform dispersion of the nanoparticles.
- The polydispersity index is determined using non-invasive back scatter technology which allows sample measurement in the range of 0.6nm-6 µm. Freshly prepared nanoparticle dispersion is placed in a folded capillary cell with/without dilution. The measurement is carried out using 4MW He-Ne laser as light source at a fixed angle of 173° at a medium temperature of 25°C.

3) Zeta potential

The Zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles [42].

- Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles.
- The Zeta potential can also be used to determine whether a charged active material is encapsulated within the center of a nanocapsule or adsorbed onto its surface.
- The charge of the nanoparticle surface is usually evaluated by the measurement of their zeta potential, which gives information about the overall surface charge of the particles and how it is affected by changes in the environment[43].
- Zeta potential is affected by the surface composition of the nanoparticles, the presence or the absence of adsorbed compounds, and the composition of the dispersing phase, mainly the Ionic strength and the pH.
- Zeta potential is measured by electrophoresis and is expressed as electrophoretic mobility or converted to the zeta potential (mV). Zeta potential measurement is done with a combination of Laser Doppler Velocimetry (LDV) and phase analysis light scattering using Zetasizer Nano.

4) Entrapment efficiency

- The capacity of the polymeric nanoparticles to entrap drug molecules into their matrix is measured in terms of entrapment efficiency.

- Entrapment efficiency is expressed in percentage (%) and is given by the equation:

$$Entrapmentefficiency(\%) = \frac{Amountofdrugwithinthenanoparticle}{Amountofdrugaddedinitially} \times 100$$

- Entrapment efficiency thus establishes a degree of how efficiently the polymeric nanoparticles are able to entrap the drug molecules within its matrix, out of the total amount of the drug added initially, during the preparation of the nanoparticles.

5) Percentage yield

- The percentage yield value is expressed in percentage (%) and is a measure of the amount of the freeze dried nanoparticles obtained, out of the total amount of the raw ingredients required to prepare the same.

  The formula used for finding out the percentage yield of a given formulation is:

$$\%yield = \frac{W1}{W2} \times 100$$

Where, $W1$= Weight of the freeze dried nanoparticles

$W2$= Weight of the initial dry weight of the starting materials (polymer + drug + crosslinking agents)

6) Fourier transform infrared spectroscopy (FT-IR)

- FT-IR studies reveal changes in chemical structure on the basis of characteristic peaks of functional groups present in the material of analysis.
- FT-IR studies are of paramount interest to detect compatibility between the drug and its excipients. Changes in peaks, formation of new peaks and disappearance of peaks are studied when doing a comparative study between the FT-IR of raw materials required for preparing the nanoparticles and the prepared nanoparticles itself. FT-IR studies also help in confirming formation of nanoparticles on the basis of several comparative studies of FT-IR of the raw materials and the nanoparticle freeze dried formulations.

7) Scanning Electron Microscopy (SEM)

- Scanning election microscope (SEM) is a type of electron microscope that is capable of producing high-resolution images, of a sample surface.
- SEM images have a characteristic three-dimensional appearance and are useful for judging the surface structure of the sample.
- Accelerated electrons in a SEM carry significant amount of kinetic energy. This energy is dissipated as signals when the incident electrons are decelerated in the solid sample. The intensity distributions of these signals give the image profile of the sample.

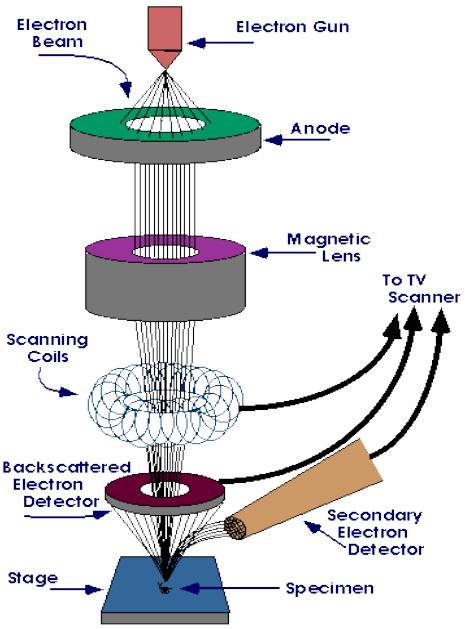


Fig 1.7.Schematic diagram of SEM.

8) Transmission Electron Microscopy (TEM)

- Transmission electron microscope (TEM) is a microscopy technique where a beam of electrons is transmitted through an ultrathin specimen (less than 200 mm).
- An image is formed from the interaction of the electrons which is then magnified and focused onto an imaging device, such as a fluorescent screen or on a layer of film, or detected by a sensor or a camera.

- Transmission electron microscopy is usually applied to nanoparticles after negative staining with phosphotungstate acid or with uranyl acetate after it has been checked that the staining agents do not modify the particles.
- Recent progress in transmission electron microscopy now allows direct observations of the nanoparticles without the use of any staining agent that may cases artefacts in some cases.

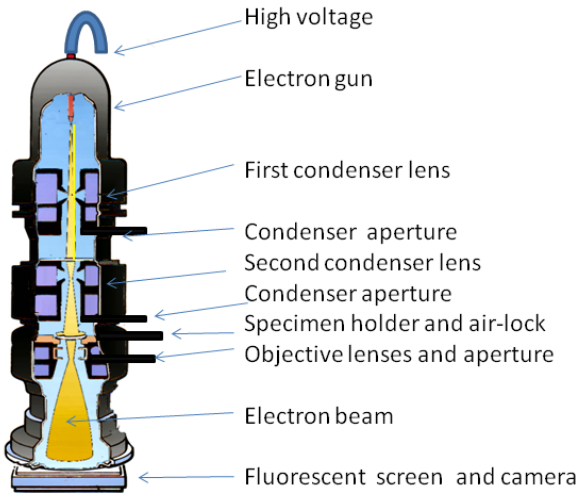


Fig 1.7.Schematic diagram of TEM.

9) *In-vitro* release

- Various methods can be used to study the *in-vitro* release of the drug [44]

a) Side -by-side diffusion cells with artificial or biological membranes.

b) Dialysis bag diffusion technique.

c) Reverse dialysis bag technique.

d) Agitation followed by ultra-centrifugation or centrifugation.

e) Ultra-filtration or centrifugal ultra-filtration techniques.

- The *in-vitro* release of drug from the nanoparticles are calculated against time. The cumulative percentage release of the drug from the nanoparticles are plotted against time, to understand the release profile of the drug from the nanoparticles.

- *In-vitro* drug release from the nanoparticles may be evaluated in phosphate buffer by using Franz Diffusion cell, where the donor chamber is filled with nanoparticle dispersion, the receptor compartment is filled with the butter solution and a dialysis membrane is fixed between the donor and the receptor chamber.

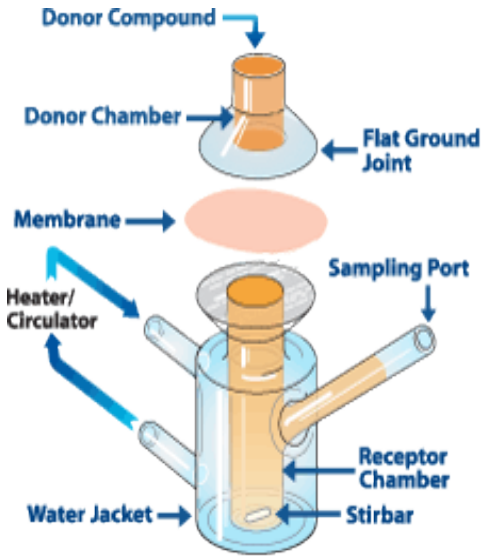


Fig 1.8.Schematic diagram of Franz Diffusion cell.

10) Drug Release

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on:(1) solubility of the drug; (2) desorption of the surface bound/adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and a better-sustained release characteristics [45].If the nanoparticle is coated by a polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes the determining factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxiliary ingredients. When the drug is involved in interaction with auxiliary ingredients to

form a less water-soluble complex, then the drug release can be very slow with almost no burst release effect [46].

11) Hydrophobic interaction chromatography

- The hydrophilicity of the nanoparticle surface can be evaluated by hydrophobic interaction chromatography.
- This technique, based on affinity chromatography allows a very rapid discrimination between hydrophilic and hydrophobic nanoparticles.
- The nanoparticles are passed through a column containing a hydrophobic interaction chromatography gel. The nanoparticles that are retained by the gel and only eluted after the addition of a surfactant are considered as hydrophobic, whereas the nanoparticles that do not interact with the gel and that are directly eluted from the column are considered as hydrophilic [43].

12) X-ray photoelectron spectroscopy (ESCA)

- ESCA is used to determine the chemical composition of the nanoparticle surface.
- This technique is a very useful tool for the development of surface modified nanoparticles providing a direct evidence of the presence of the components that are believed to be on the nanoparticle surface [43].

CHAPTER 2:
REVIEW OF LITERATURE

2. Review of literature

- Fan and coworkers developed monodisperse, low molecular weight (LMW) chitosan nanoparticles by a novel method based on ionic gelation using sodium tripolyphosphate (TPP) as cross-linking agent. The focus of this study was to solve the problem of preparation of chitosan/TPP nanoparticles with high degree of monodispersity and stability, and investigate the effect of various parameters on the formation of LMW chitosan/TPP nanoparticles. It was found that the particle size distribution of the nanoparticles could be significantly narrowed by a combination of decreasing the concentration of acetic acid and reducing the ambient temperature during cross-linking process. The optimized nanoparticles exhibited a mean hydrodynamic diameter of 138 nm with a polydispersity index (PDI) of 0.026 and a zeta potential of +35 mV, the nanoparticles had good storage stability at room temperature up to at least 20 days (Fan *et al*., 2012).

- Tsai and coworkers investigated the effect of initial size and pH of the solution on the changes in size of chitosan/tripolyphosphate (CS/TPP) nanoparticles stored in a phosphate buffer at 25 °C. The size decreased with increasing pH of the storage phosphate buffer. The initial sizes of the nanoparticles themselves also affected storage stability—the larger ones decreased in size, however the smaller ones increased their size in a phosphate buffer with a pH of 7.5 at 25 °C for 10 days due to protonation or deprotonation effects on the chitosan molecules. The changes of nanoparticle sizes are classified into instantaneous, aging, and swelling/aggregation stages over the storage time of 97 days (Tsai *et al*, 2012).

- Trapani and coworkers developed and characterize nanoparticles (NPs) composed of chitosan (CS) and evaluated their potential for brain delivery of the neurotransmitter Dopamine (DA). For this purpose, CS based NPs were incubated with DA at two different concentrations giving rise to nanocarriers denoted as DA/CSNPs (1) and DA/CSNPs (5), respectively. X-ray Photoelectron Spectroscopy (XPS) analysis

- confirmed that DA was adsorbed onto the external surface of such NPs. The cytotoxic effect of the CSNPs and DA/CSNPs was assessed using the MTT test and it was found that the nanovectors are less cytotoxic than the neurotransmitter DA after 3h of incubation time. Transport studies across MDCKII-MDRl cell line showed that DA/CSNPs (5) give rise to a significant transport enhancing effect compared with the control and greater than the corresponding DA/CSNPs (1). Measurement of reactive oxygen species (ROS) suggested a low DA/CSNPs neurotoxicity after 3 h. *In-vivo* brain micro dialysis experiments in rat showed that intra-peritoneal acute administration of DA/CSNPs (5) (6-12 mg/kg) induced a dose-dependent increase in striatal DA output. Thus, these CS nanoparticles represent an interesting technological platform for DA brain delivery and, hence, may be useful for Parkinson's disease treatment (Trapani *et al.,* 2011)

- Arjun and co-workers designed and developed chitosan nanoparticles which were loaded with two different drugs simultaneously. Aspirin (acetylsalicylic acid, ASA), a hydrophilic drug and probucol (PRO), a hydrophobic drug, were chosen, which are widely used to treat restenosis. The drug loaded chitosan nanoparticles were prepared by gelation of chitosan with tripolyphosphate (TPP) by ionic cross-linking. The physicochemical properties of nanoparticles were investigated by FTIR, transmission electron microscope (TEM), scanning electron microscopy (SEM) and differential scanning calorimetry (DSC). The images showed that these particles were spherical in shape with ASA being in the amorphous phase, while PRO being crystalline. The properties of chitosan nanoparticles such as encapsulation capacity and controlled release behaviors of ASA and PRO were evaluated. Experimental results indicated that the loading capacity (LC), encapsulation efficiency (EE) and ASA and PRO release behaviors were affected by several factors including pH, concentration of TPP, chitosan molecular weight (MW) and ASA initial concentration as well as PRO. In vitro release showed that the nanoparticles provided a continuous release. Entrapped ASA was released for more than 24 h and PRO lasts longer for 120 h (Arjun *et al*., 2009).

- Liu and Gao fabricated chitosan nanoparticles by a method of tripolyphosphate (TPP) cross-linking. The influence of fabrication conditions on physical properties, drug loading and release properties were investigated by transmission electron microscopy (TEM), dynamic light scattering (DLS), and UV-VIS spectroscopy. The nanoparticles could be prepared only within a zone of appropriate chitosan and TPP concentrations. The particle size and surface zeta potential can be manipulated by variation of the fabrication conditions such as chitosan/TPP ratio and concentration, solution pH and salt addition. TEM observation revealed a core-shell structure for the prepared nanoparticles, but a filled structure for the ciprofloxacin (CH) loaded particles. The results showed that the chitosan nanoparticles were rather stable and no cytotoxicity of the chitosan nanoparticles was found in *in-vitro* cell culture experiment. Loading and release of CH can be modulated by the environmental factors such as solution pH and medium quality (Liu & Gao., 2009).
- Wu and associates have prepared ammonium glycyrrhizinate-loaded chitosan nanoparticles by ionic gelation of chitosan with tripolyphosphate anions (TPP). The particle size and zeta potential of nanoparticles were determined by dynamic light scattering (DLS) and a zeta potential analyzer respectively. The effect studied included chitosan molecular weight, chitosan concentration, ammonium glycyrrhizinate concentration and polyethylene glycol (PEG) on the physicochemical properties of the nanoparticles. These nanoparticles achieved a good loading efficiency of ammonium glycyrrhizinate. It was observed that the encapsulation efficiency decreased with the increase of ammonium glycyrrhizinate concentration. The introduction of PEG could decrease significantly the positive charge of particle surface. Thus, these studies showed that chitosan form complex with TPP to formed stable cationic nanoparticles (Wu *et al*., 2005).
- Chenguang and coworkers reviewed the methods of preparation for chitosan-based nanoparticles and their pharmaceutical applications. Namely emulsion cross-linking, emulsion-droplet coalescence, ionic gelation, reverse micellar method and chemically modified chitosan method. Chitosan nanoparticles are used as earners for low molecular weight drug, vaccines and DNA (Chenguang *et al*., 2006).

CHAPTER 3:
AIMS AND OBJECTIVES

3. Aims and Objectives

The present research work encompasses the development of Capecitabine loaded chitosan nanoparticles, by the Ionotropic gelation method, using Sodium tripolyphosphate as the crosslinking agent. Capecitabine is a very potent anti-cancer drug and may pose a threat of being toxic upon repeated administrations. Conventional oral dosage forms like tablets or capsules, often leads to poor bioavailability of Capecitabine, due to first-pass metabolism (FPM).Nanoparticle based drug delivery is a more suitable form of anti-cancer drug delivery because of its targeted action, more effective therapeutic activity by utilizing a very low dose of the drug and reduction in the frequency of administration.

In this present study the objective includes:

1. Assessment of drug-excipient physical interaction by visual inspection.
2. Assessment of the drug-excipient compatibility by FT-IR analysis and DSC analysis.
3. Fabrication of Capecitabine loaded chitosan nanoparticles by Ionotropic gelation method.
4. Optimization of the Capecitabine loaded nanoparticle formulation by varying the chitosan concentration.
5. Characterization by:
    - Particle size analysis, Polydispersity index and zeta potential using particle size analyzer.
    - Entrapment efficiency of the prepared Capecitabine loaded nanoparticle formulations.
    - Percentage Yield of the prepared Capecitabine loaded nanoparticle formulations.
    - Fourier transform infra-red spectroscopy (FT-IR) of the blank nanoparticles and of the optimized Capecitabine loaded nanoparticle formulation.
    - Differential Scanning Calorimetry (DSC) of the blank nanoparticles and of the optimized Capecitabine loaded nanoparticle formulation.
    - Scanning Electron Microscopy (SEM) of the prepared Capecitabine loaded nanoparticle formulations.

- Transmission Electron Microscopy (TEM) of the prepared Capecitabine loaded nanoparticle formulations.
- *In Vitro* drug release studies of the prepared Capecitabine loaded nanoparticle formulations by dialysis bag diffusion technique.
- Drug release kinetic studies of the prepared Capecitabine loaded nanoparticle formulations.

CHAPTER 4:

MATERIALS AND METHODS

4. Materials and Methods

4.1. Materials

4.1.1. Chemicals and Reagents used:

- Capecitabine was obtained as a gift sample from Cipla Pvt. Ltd., Sikkim, India.
- LMW chitosan (degree of deacetylation ≥ 75.00%) was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India.
- Sodium tripolyphosphate (STPP) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India.
- Glacial acetic acid was purchased from Merck Specialities Pvt. Ltd., Mumbai, India.
- Sodium hydroxide was purchased from Merck Specialities Pvt. Ltd., Mumbai, India.
- Potassium chloride was purchased from Process Chemical Industries, Kolkata, India.
- Hydrochloric acid 37% EMPARTA® ACS analytical reagent was purchased from Merck Specialities Pvt. Ltd., Mumbai, India.
- Potassium dihydrogen phosphate was purchased from Merck Specialities Pvt. Ltd.,Mumbai, India.
- Sodium chloride was purchased from Process Chemical Industries, Kolkata, India.
- Di-sodium hydrogen phosphate was purchased from Process Chemical Industries, Kolkata, India.

  All other reagents were of analytical grade.

4.1.2. Analytical Facilities and Equipments used:

- Electronic balance (Precisa 310M)
- pH Meter (Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer)
- Ultrapure/ Milli-Q water purification unit (Arium® pro,Sartorius)
- Magnetic stirrer (2MLH, Remi Equipment Pvt.Ltd., India)
- Probe-sonicator (LABSONIC®M, Ultrasonic homogeniser, Sartorius)
- Biofreezer(Labtop Instruments Pvt. Ltd.)
- Lyophilizer (Scanvac,Coolsafe 55-4)
- Humidity chamber (Instrumentation India, Kolkata)

- Cooling Ultra-Centrifuge (SIGMA 3K30 Laboratory Centrifuge)
- UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan)
- Dynamic light scattering (Malvern Zetasizer, NanoZS, Model no. ZEN3500: Malvern Instruments, Malvern, Worcestershire, UK)
- Fourier transform infra-red spectroscope (IR- Prestige-21Shimadzu make, Japan)
- Differential Scanning Calorimeter (Pyris Diamond TG/DTA, PerkinElmer, SINGAPORE).
- Scanning electron microscope (Zeiss EVO®18)
- Transmission electron microscope(JEM 2100,200 KV HRTEM,Jeol make)

4.1.3. Drug Profile

Capecitabine is an orally administered chemotherapeutic agent, used in the treatment of various tumor types including metastatic breast and colorectal cancer and is commercially available as an immediate release tablet (Xeloda®,Roche)[47].Capecitabine is a pre-pro-drug that is enzymatically converted into 5-Fluorouracil(5-FU)in the tumor,where it inhibits DNA synthesis and slows growth of tumor tissue.Capecitabine increases the concentration of the active principle at the tumor site and has greater activity.The concentration of Capecitabine is reduced in healthy tissues with a consequent reduction in systemic toxicity.

4.1.3.1. Structural formula:

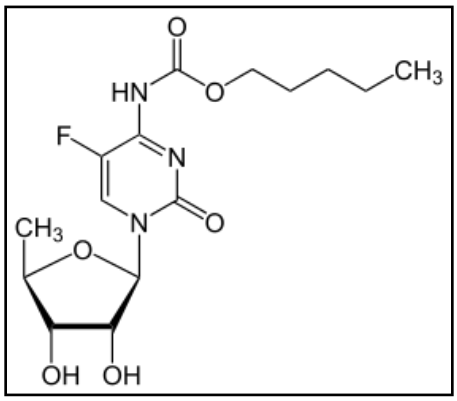


Fig 4.3.Structure of Capecitabine.

4.1.3.2. IUPAC Name: Pentyl [1-(3,4-dihydroxy-5-methyltetrahydrofuran-2-yl)-5-fluoro-2-oxo-1*H*-pyrimidin-4-yl]carbamate

4.1.3.3. Synonym: N(4)-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine

4.1.3.4. Molecular formula: $C_{15}H_{22}FN_3O_6$.

4.1.3.5. Molecular weight:359.350083g/mol.

4.1.3.6. Formal Charge: 0.

4.1.3.7. BCS Class: I.

4.1.3.8. Therapeutic Class: Anticancer, Antimetabolite.

4.1.3.9. Pharmacological Class: Pyrimidine analogue

4.1.3.10. Appearance: White to off-white crystalline powder.

4.1.3.11. Melting Point: 110-121 °C

4.1.3.12. Solubility: Sparingly soluble in water with a maximum solubility of 26 mg/ml at 20°C, freely soluble in anhydrous ethanol.

4.1.3.13. pKa value of Capecitabine: pKa (Strongest Acidic) :8.23;pKa (Strongest Basic):-3.6

4.1.3.14. Biological Half-Life:45-60 minutes

4.1.3.15. logP value: Capecitabine:0.97 ; 5-FU: - 0.78

4.1.3.16.Plasma protein binding: Plasma protein binding (mainly to albumin) of Capecitabine and its metabolites is less than 60% and is not concentration dependent.

4.1.3.17. Storage: Capecitabine should be stored at a temperature not exceeding 30°C

4.1.3.18. Stability: Capecitabine tablets are stable for at least 9 months when stored in tightly closed containers at room temperature [48].

4.1.3.19. Dose of Capecitabine: The approved dose (2500 mg/m^2/day as intermittent regimen) leads to unacceptable toxicity in many patients with metastatic breast cancer. A recent retrospective analysis suggests a standard starting dose of 2000 mg/ m^2/day. If patients still have toxicity , individualization of dosing is necessary [49].

4.1.3.20. Dosage regimen of Capecitabine: 1250 mg/m^2 twice daily (morning-evening) oral dosing.

4.1.3.21. FDA Pregnancy Risk category: D

4.1.3.22.Pharmacology:

a) **Mechanism of action:** After oral administration, Capecitabine crosses the gastrointestinal barrier intact and is rapidly and almost completely absorbed [50-51]. It is subsequently converted into 5-Fluorouracil (FU) in a three stage mechanism involving several enzymes. In the first step, it is metabolized into 5′- deoxy -5- fluorocytidine (5′dFCR) by hepatic carboxylesterase, which is then deaminated into 5′- deoxy - 5 - fluorouridine (5′dFUrd) by cytidine deaminase, mainly localized in tumor tissues. Finally 5′dFUrd is transformed into FU under the action of thymidine phosphorylase (TP), an enzyme with higher activity in tumor cells than in normal tissues[52].

b) **Contraindications**

- History of severe and unexpected reactions to fluoropyrimidine therapy,
- Hypersensitivity to Capecitabine or to any of the excipients listed in section 6.1 or fluorouracil,
- In patients with known complete absence of dihydropyrimidine dehydrogenase (DPD) activity,
- During pregnancy and lactation,
- In patients with severe leukopenia, neutropenia or thrombocytopenia,
- In patients with severe hepatic impairment,
- In patients with severe renal impairment (creatinine clearance below 30 ml/min),

c) Drug warning:

- **Dose limiting toxicities:** Diarrhoea, abdominal pain, nausea, stomatitis and hand-foot syndrome (hand-foot skin reaction, palmer-plantar erythrodysesthesia). Most adverse reactions are reversible and do not require permanent discontinuation of therapy, although doses may need to be withheld or reduced.
- **Diarrhoea:** Patients with severe diarrhoea should be carefully monitored and given fluid and electrolyte replacement if they become dehydrated. Standard antidiarrhoeal treatments (e.g. loperamide) may be used. Dose reduction should be applied as necessary.
- **Hand-foot syndrome (also known as hand-foot skin reaction or palmar-plantar erythrodysesthesia or chemotherapy induced acral erythema):** Grade 1 hand-foot syndrome is defined as numbness, dysesthesia/paresthesia, tingling, painless swelling or erythema of the hands and/or feet and/or discomfort which does not disrupt the patient's normal activities. Grade 2 hand-foot syndrome is painful erythema and swelling of the hands and/or feet and/or discomfort affecting the patient's activities of daily living. Grade 3 hand-foot syndrome is moist desquamation, ulceration, blistering and severe pain of the hands and/or feet and/or severe discomfort that causes the patient to be unable to work or perform activities of daily living. If grade 2 or 3 hand-foot syndrome occurs, administration of Capecitabine should be interrupted until the event resolves or decreases in intensity to grade 1.
- **Cardiotoxicity:** Cardiotoxicity has been associated with fluoropyrimidine therapy, including myocardial infarction, angina, dysrhythmias, cardiogenic shock, sudden death and electrocardiographic changes (including very rare cases of QT prolongation). These adverse reactions may be more common in patients with a prior history of coronary artery disease. Cardiac arrhythmias (including ventricular fibrillation, torsades de pointes, and bradycardia), angina pectoris, myocardial infarction, heart failure and cardiomyopathy have been reported in patients receiving Capecitabine. Caution must be exercised in patients with history of significant cardiac disease, arrhythmias and angina pectoris.
- **Central or peripheral nervous system diseases:** Caution must be exercised in patients with central or peripheral nervous system diseases, e.g. brain metastasis or neuropathy.
- **Diabetes mellitus or electrolyte disturbances:** Caution must be exercised in patients

with diabetes mellitus or electrolyte disturbances, as these may be aggravated during Capecitabine treatment[53].

4.1.3.23. Absorption, Distribution, Metabolism and Elimination:

i. Following oral administration of Capecitabine 1250 mg/m² BID to cancer patients, Capecitabine reached peak blood levels in about 1.5 hours (t_{max}) with peak 5-FU levels (C_{max})occurring slightly later, at 2 hours. Capecitabine is readily absorbed through the GI tract (~70%).

ii. An activation pathway of Capecitabine has been proposed that requires three sequential steps of enzyme reactions (Bajetta et al., 1996). Capecitabine is first converted to 5′-deoxy-5-fluorocytidine (5′-DFCR) by carboxylesterase and then to 5′-DFUR by cytidine deaminase, mainly in the liver (Budman et al., 1998; Miwa et al., 1998). Finally, systemic 5′-DFUR is converted to 5-FU by thymidine phosphorylase, the activity of which is increased in tumor tissues.

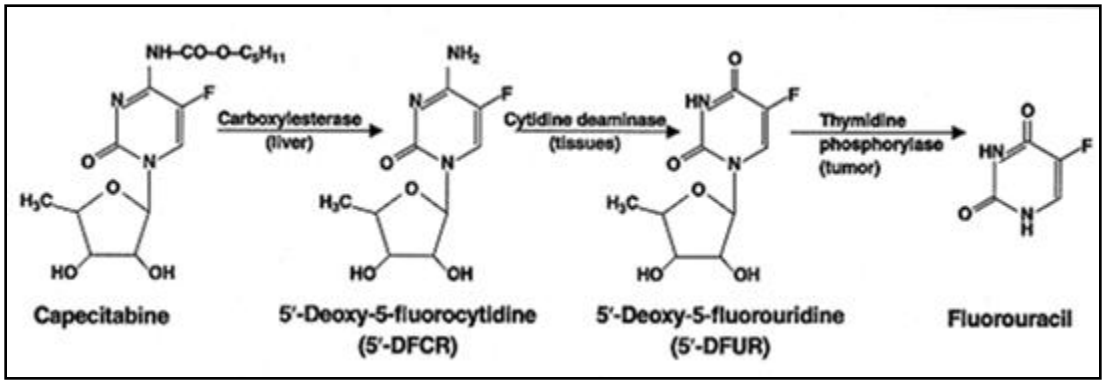


Fig 4.2: Schematic representation of Capecitabine metabolism.

iii. Plasma protein binding of Capecitabine and its metabolites is less than 60% and is not concentration-dependent. Capecitabine was primarily bound to human albumin (approximately 35%).

iv. Capecitabine and its metabolites are predominantly excreted in urine; 95.5% of administered Capecitabine dose is recovered in urine. Fecal excretion is minimal (2.6%).

v. The major metabolite excreted in urine is alpha-fluoro-beta-alanine (FBAL) which

represents 57% of the administered dose. About 3% of the administered dose is excreted in urine as unchanged drug [54].

4.1.4. Polymer Profile

Chitosan is derived from naturally occurring sources, which is the exoskeleton of insects, crustaceans and fungi that has been shown to be biocompatible and biodegradable [55].Chitosan is a polyelectrolyte with reactive functional groups, gel-forming capability, high adsorption capacity and biodegradability. Chitosan is a linear copolymer of β-(1-4) linked 2-acetamido-2-deoxy-β–D-glucopyranose and 2-amino-2-deoxy-β–D-glycopyranose. It is a polycationic polymer that has one amino group and two hydroxyl groups in the repeating glucosidic residue. Chitosan exists under many different chemical structures, being available either as a base or a salt (such as chloride, lactate, glutamate etc.).

Chitosan is a linear polysaccharide derived from naturally occurring Chitin composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. Chitosan is obtained by the thermo-chemical deacetylation of chitin in the presence of alkali and naturally it occurs only in certain fungi(Mucoraceae) [56].The treatment of chitin with an aqueous 40-45% (w/v) NaOH solution at 90-120 °C for 4-5h results in N-deacetylation of chitin.

Ionic complexes are readily formed by ionic interactions between the cationic chitosan and negatively charged molecules, such as sulfates, citrates and phosphate ions [57].The active primary amino groups on the molecule being reactive provides sites for a variety of side group attachment employing mild reaction conditions. At low pH, these amines get protonated and become positively charged which makes chitosan a water-soluble cationic polyelectrolyte. On the other hand, as the pH increases above 6, chitosan's amines become deprotonated and the polymer loses its charge and becomes insoluble.

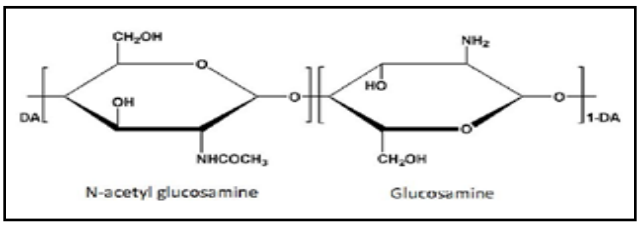


Fig 4.4. Structure of chitosan.

Calvo *et al*. (1997) had developed a technique for developing chitosan nanogels by adding a cross linking agent tripolyphosphate (TPP) into the aqueous phase containing chitosan.

Chitosan has attracted attention as an excellent mucoadhesive in its swollen state and a natural bioadhesive polymer that can adhere to hard and soft tissues. The hydrogen bonding and ionic interactions are responsible for the adhesive properties of chitosan and different substrates. The amine groups of chitosan serve for covalent attachment of biomolecules and it can be co-deposited with other polymers or nanoparticles.

Chitosan offers several advantages, and these include its ability to control the release of active agents and avoid the use of hazardous organic solvents while fabricating particles since it is soluble in aqueous acidic solution. Chitosan has favorable biocompatibility characteristics as well as the ability to increase membrane permeability, both *in vitro* and *in vivo* and can be degraded by lysozyme in serum. Chitosan's pH dependent solubility allows them to be extensively studied for delivery of anticancer agents, therapeutic proteins, genes and antigens. Chitosan can form stable films on various surfaces under neutral and basic pH conditions. Hence chitosan can be used for controlled drug release, wound healing, nutrition supplements, water purification, removal of toxins etc. Chitosan has been used for the development of drug delivery systems with a regulated drug release rate and a reduced frequency of administration of the drug due to its gel-forming ability in low pH range. In recent years, low molecular weight chitosan nanoparticles have shown great potential in the application of drug delivery and non-viral vector for gene delivery.

4.2. Methods

4.2.1. Drug- Excipient interaction study

To know the interaction between Capecitabine and other excipients like chitosan, sodium tripolyphosphate, gelatin, glutaraldehyde, HPMC, ethanol, acetone, carbopol and CAP, all were kept in humidity chamber (Instrumentation India, Kolkata) at 40°C and 75% relative humidity for 6 months. By visual inspection, change of colour and the change of state, were found out.

4.2.2. Drug - Excipient compatibility study

The FT-IR spectral analysis was carried out for pure Capecitabine, excipient blend (LMW chitosan and sodium tripolyphosphate) and for a physical mixture of Capecitabine with the excipients, by using IR- Prestige-21Shimadzu make, Japan. The analysis was performed twice. The initial study was done at the beginning of the experiment and the final one was done after storing the samples in the humidity chamber (Instrumentation India, Kolkata) at 40±2°C and 75 ± 5%RH for a period of 6 months .Each of the samples were crushed with KBr (1%w/w nanoparticles) to form pellets by applying pressure at 600 kg/cubic cm and were scanned between 400-4500 cm^{-1} with a resolution of $4cm^{-1}$ and at a speed of 2mm/second.

4.2.3. Characterization of Capecitabine:

4.2.3.1. Determination of maximum absorbance of Capecitabine and preparation of calibration curve:

a) Ultraviolet spectroscopy

A specific amount of Capecitabine was dissolved in a certain volume of Milli-Q water, 0.1 M hydrochloric acid (pH 1.2), phosphate buffer pH 6.8 and phosphate buffer pH 7.4, to prepare the stock solution of Capecitabine in various pH. The different concentrations of the Capecitabine solution obtained after appropriate dilution with the respective buffer solutions were subjected to UV- Spectrophotometric analysis, for determining the absorption maxima (λ_{max}) of Capecitabine. Each of the diluted concentrations were scanned in the range of 200 to 400 nm using UV-Visible Spectrophotometer, UV-2450 (Shimadzu, Japan).

b) Preparation of 0.1 M hydrochloric acid solution, pH 1.2

Exactly 8.3 ml of concentrated hydrochloric acid solution (37%) was added to 1000 ml of distilled water, in a 1000 ml volumetric flask. The pH was measured using Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer.

c) Preparation of Phosphate Buffer pH 6.8 IP

28.80 g of disodium hydrogen phosphate and 11.45 g of potassium dihydrogen phosphate were dissolved in sufficient amount of distilled water to produce 1000ml.The pH was measured using Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer.

d) Preparation of Phosphate Buffer pH 7.4 IP

To 50ml of 0.2M potassium dihydrogen phosphate, exactly 39.1 ml of 0.2M sodium hydroxide was added. The volume was made upto 200 ml with distilled water. The pH was measured using Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer.

e) Determination of λ_{max} of Capecitabine in Milli-Q water

A stock solution of Capecitabine was prepared by accurately weighing 10 mg of Capecitabine in an analytical balance (Precisa 310M) and dissolving it in 100 ml of Milli-Q water (Arium® pro, Sartorius), to get a concentration of 0.1mg/ml. From the prepared stock solution of Capecitabine, 0.5ml was withdrawn and placed in a 10 ml volumetric flask. Milli-Q water was added to make up the volume upto 10 ml. The resulting solution (0.005 mg/ml) was scanned in UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan), in the range of 400 to 200 nm, to obtain the spectrum of Capecitabine and hence its λ_{max}.

f) Determination of λ_{max} of Capecitabine in 0.1 M hydrochloric acid (pH 1.2)

A stock solution of Capecitabine was prepared by accurately weighing 10 mg of Capecitabine in an analytical balance (Precisa 310M) and dissolving it in 100 ml of 0.1 M hydrochloric acid (pH 1.2), to get a concentration of 0.1mg/ml. From the prepared stock solution of Capecitabine, 0.5ml was withdrawn and placed in a 10 ml volumetric flask. The same acid solution was added to make up the volume upto 10 ml. The resulting solution (0.005 mg/ml) was scanned in UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan), in the range of 400 to 200 nm, to obtain the spectrum of Capecitabine and hence its λ_{max}.

g) Determination of λ_{max} of Capecitabine in Phosphate Buffer pH 6.8

From the prepared stock solution of Capecitabine, 0.5ml was withdrawn and placed in a 10ml volumetric flask. The same buffer solution was added to make up the volume upto 10 ml.

The resulting solution (0.005 mg/ml) was scanned in UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan), in the range of 400 to 200 nm, to obtain the spectrum of Capecitabine and hence its λ_{max}.

h) Determination of λ_{max} of Capecitabine in Phosphate Buffer pH 7.4

A stock solution of Capecitabine was prepared by accurately weighing 10 mg of Capecitabine in an analytical balance (Precisa 310M) and dissolving it in 100 ml of Phosphate Buffer pH 7.4, to get a concentration of 0.1mg/ml. From the prepared stock solution of Capecitabine, 0.5ml was withdrawn and placed in a 10 ml volumetric flask. The same buffer solution was added to make up the volume upto 10 ml. The resulting solution (0.005 mg/ml) was scanned in UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan), in the range of 400 to 200 nm, to obtain the spectrum of Capecitabine and hence its λ_{max}.

i) Calibration curve of Capecitabine in Milli-Q water

Aliquots of 0.5 ml, 1ml, 2 ml, 3 ml, 4 ml and 5 ml were withdrawn from the prepared stock solution of Capecitabine in Milli-Q water and were diluted to 10 ml by Milli-Q water to obtain Capecitabine solutions, with a concentration in the range of 0.005 mg/ml to 0.05 mg/ml. Absorbance of these solutions were measured at 215 nm, in UV-Visible Spectrophotometer, UV-2450 (Shimadzu, Japan), using Milli-Q water as blank. The calibration curve of Capecitabine was plotted, taking the concentration values in mg/ml on the X-axis and absorbance values on the Y-axis on an MS-excel data sheet.

j) Calibration curve of Capecitabine in 0.1 M hydrochloric acid (pH 1.2)

Aliquots of 0.5 ml, 1ml, 2 ml, 3 ml, 4 ml and 5 ml were withdrawn from the prepared stock solution of Capecitabine in 0.1 M hydrochloric acid (pH 1.2) and were diluted to 10 ml by the same acid solution to obtain Capecitabine solutions, with a concentration in the range of 0.005 mg/ml to 0.05 mg/ml. Absorbance of these solutions were measured at 215 nm, in UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan), using 0.1 M hydrochloric acid as blank. The calibration curve of Capecitabine was plotted, taking the concentration values in mg/ml on the X-axis and absorbance values on the Y-axis on an MS-excel data sheet.

k) Calibration curve of Capecitabine in Phosphate Buffer pH 6.8

Aliquots of 0.5 ml, 1ml, 2 ml, 3 ml, 4 ml and 5 ml were withdrawn from the prepared stock solution of Capecitabine in Phosphate Buffer pH 6.8 and were diluted to 10 ml by the same buffer solution to obtain Capecitabine solutions with a concentration in the range of 0.005 mg/ml to 0.05 mg/ml. Absorbance of these solutions were measured at 215 nm, in UV-Visible Spectrophotometer, UV-2450 (Shimadzu, Japan), using Phosphate Buffer pH 6.8 as blank. The calibration curve of Capecitabine was plotted, taking the concentration values in mg/ml on the X-axis and absorbance values on the Y-axis on an MS-excel data sheet.

l) Calibration curve of Capecitabine in Phosphate Buffer pH 7.4

Aliquots of 0.5 ml, 1ml, 2 ml, 3 ml, 4 ml and 5 ml were withdrawn from the prepared stock solution of Capecitabine in Phosphate Buffer pH 7.4 and were diluted to 10 ml by the same buffer solution to obtain Capecitabine solutions with a concentration in the range of 0.005 mg/ml to 0.05 mg/ml. Absorbance of these solutions were measured at 215 nm, in UV-Visible Spectrophotometer, UV-2450 (Shimadzu, Japan), using Phosphate Buffer pH 7.4 as blank. The calibration curve of Capecitabine was plotted, taking the concentration values in mg/ml on the X-axis and absorbance values on the Y-axis on an MS-excel data sheet.

4.2.3.2. Fourier transform infra-red (FT-IR) spectroscopy

The FTIR spectral analysis was carried out for pure Capecitabine by using IR- Prestige-21Shimadzu make, Japan. Approximately 2 mg of Capecitabine was crushed with KBr (1%w/w nanoparticles) to form pellets by applying pressure at 600 kg/cubic cm and were scanned between 400-4500 cm^{-1} with a resolution of $4cm^{-1}$ and at a speed of 2mm/second.

4.2.3.3. Differential Scanning Calorimetry (DSC)

DSC was performed for pure Capecitabine using Pyris Diamond TG/DTA, PerkinElmer (SINGAPORE).About 20 mg of the sample was placed on a standard shallow platinum crucible. Platinum crucible with alpha alumina powder was used as the reference. The sample cell was heated at a uniform scan rate of 15°C/min from 30°C to 1000°C under a constant nitrogen purge

of 150ml/min .Heat flow in mW was obtained as a function of sample temperature in degrees centigrade.

4.2.4. Characterization of Chitosan:

4.2.4.1. Fourier transform infra-red (FT-IR) spectroscopy

The FTIR spectral analysis was carried out for low molecular weight (LMW) chitosan by using IR- Prestige-21Shimadzu make, Japan. About 2 mg of LMW chitosan were crushed with KBr (1%w/w nanoparticles) to form pellets by applying pressure at 600 kg/cubic cm and were scanned between 400-4500 cm^{-1} with a resolution of $4cm^{-1}$ and at a speed of 2mm/second.

4.2.4.2. Differential Scanning Calorimetry (DSC)

DSC was performed for LMW chitosan using Pyris Diamond TG/DTA, PerkinElmer (SINGAPORE).About 20 mg of chitosan was placed on a standard shallow platinum crucible. Platinum crucible with alpha alumina powder was used as the reference. The sample cell was heated at a uniform scan rate of 15°C/min from 30°C to 1000°C under a constant nitrogen purge of 150ml/min .Heat flow in mW was obtained as a function of sample temperature in degrees centigrade.

4.2.5. Preparation of chitosan nanoparticles:

Chitosan-tripolyphosphate nanoparticles were prepared by Ionotropic gelation method (Calvo et al., 1997). Varying amounts of low molecular weight chitosan were weighed and dissolved in 10 ml aqueous solution of 1% w/v acetic acid by constant stirring at 350 rpm at room temperature, in a magnetic stirrer (2MLH, Remi Equipment Pvt.Ltd., India). After formation of a transparent solution, the pH was adjusted to 4.7 using 0.1N NaOH solution. 2mg of sodium tripolyphosphate (TPP) and 25 mg of Capecitabine were weighed and dissolved separately in 1ml of Milli-Q water until a clear solution was obtained. For preparing blank nanoparticles, TPP solution was added drop wise to the chitosan solution, using a syringe needle under constant stirring (600 rpm) at room temperature. For preparing Capecitabine loaded nanoparticles, the Capecitabine solution was added drop wise to the chitosan solution using a syringe needle under constant stirring (600 rpm) at room temperature prior to the addition of TPP solution. Spontaneous formation of an

opalescent suspension was observed and was left for stirring at room temperature, for 1 hour. The nanoparticle suspension thus formed was sonicated (LABSONIC®M, Ultrasonic homogeniser, Sartorius) for 25 minutes and was ultra-centrifuged at 15000 rpm (SIGMA 3K30 Laboratory Centrifuge) for 20 minutes at 4°C, to remove the non-entrapped drug. The supernatant was discarded and the wet pellets of chitosan-tripolyphosphate nanoparticles were collected. The pellets were freeze-dried and stored in an air tight closed container at 2-4°C. The different formulation parameters of all the batches were tabulated in Table 4.1.

Table 4.1. Formulation parameters.

S.No	Formulations	pH	Chitosan:TPP	Drug(mg)
1	CN-1	4.7	2:1	25
2	CN-2	4.7	2.3:1	25
3	CN-3	4.7	2.6:1	25
4	CN-4	4.7	2.9:1	25
5	CN-5	4.7	3.3:1	25
6	CN-6	4.7	3.5:1	25
7	CN-7	4.7	3.8:1	25
8	CN-8	4.7	4:1	25
9	CN-9	4.7	4.5:1	25

4.2.6. Characterization of chitosan-tripolyphosphate nanoparticles:

4.2.6.1. Particle Size, polydispersity index (PDI) and zeta potential

The determination of particle size, polydispersity index (PDI) and zeta potential of Capecitabine loaded nanoparticle dispersions were carried out by dynamic light scattering using Malvern

Zetasizer (NanoZS, Model no. ZEN3500: Malvern Instruments, Malvern, Worcestershire, UK) following a 1/3 (v/v) dilution in Milli-Q water at 25°C.

4.2.6.2. Entrapment Efficiency (%)

Exactly 2ml of each Capecitabine loaded chitosan nanoparticle formulation was taken in separate Eppendorf micro centrifuge tubes of 2ml volume, with the help of a micropipette. The filled Eppendorf tubes were then subjected to centrifugation at a speed of 15000 rpm for 20 minutes at 4°C.The supernatant was collected after centrifugation and its absorbance was checked in UV-Visible Spectrophotometer, UV-2450 (Shimadzu,Japan) at 215 nm. The free drug concentration present in the supernatant was determined from the calibration curve of Capecitabine. The amount of free drug present in the supernatant was then subtracted from the amount of drug taken initially, to obtain the amount of bound drug present within the nanoparticles. For determining the entrapment efficiency of the nanoparticles, the following equation was used:

$$Entrapmentefficiency(\%) = \frac{Amountofdrugwithinthenanoparticle}{Amountofdrugaddedinitially} \times 100$$

4.2.6.3. Percentage Yield (% yield)

The percentage yield of Capecitabine loaded nanoparticle formulations were performed by taking the weight of the nanoparticles recovered after lyophilisation and dividing it by the sum of initial dry weight of the starting materials. The formula used was:

$$\%yield = \frac{W1}{W2} \times 100$$

Where, W1 = Weight of the lyophilized nanoparticles

W2 = Weight of the initial dry weight of the starting materials (Polymer+drug+STPP).

4.2.6.4. Fourier transform infra-red (FT-IR) spectroscopy

The FTIR spectral analysis was carried out for blank chitosan-tripolyphosphate nanoparticles and Capecitabine loaded nanoparticles (CN-4) by using IR- Prestige-21Shimadzu make, Japan. About 2 mg of each of the samples were crushed with KBr (1%w/w nanoparticles) to form

pellets by applying pressure at 600 kg/cubic cm and were scanned between 400-4500 cm^{-1} with a resolution of 4cm^{-1} and at a speed of 2mm/second.

4.2.6.5. Differential Scanning Calorimetry (DSC)

DSC was performed separately for blank chitosan-tripolyphosphate nanoparticles and Capecitabine loaded nanoparticles (CN-4) using Pyris Diamond TG/DTA, PerkinElmer (SINGAPORE).About 20 mg of the sample was placed on a standard shallow platinum crucible. Platinum crucible with alpha alumina powder was used as the reference. The sample cell was heated at a uniform scan rate of 15°C/min from 30°C to 1000°C under a constant nitrogen purge of 150ml/min .Heat flow in mW was obtained as a function of sample temperature in degrees centigrade.

4.2.6.6. Scanning Electron Microscopy (SEM)

The surface morphology of freeze dried Capecitabine loaded nanoparticles (CN-4 and CN-5) were determined by scanning electron microscopy technique using Zeiss EVO®18, which was operated at an accelerating voltage of 20kV.Images of the samples were captured at different magnifications.

4.2.6.7. Transmission Electron Microscopy (TEM)

The morphological characteristics of the Capecitabine loaded nanoparticles (CN-4 and CN-5) were examined using a high resolution transmission electron microscope(200 KV HRTEM, Jeol make).A droplet of nanoparticle suspension was placed on a carbon-coated copper grid of 200 mesh (TED PELLA, INC) without being stained. Five minutes later, the excess liquid was removed by touching the edged of the copper grid with a piece of filter paper. The sample was then air-dried before observation by TEM. Images were captured at different magnifications.

4.2.6.8. *In Vitro* drug release study

The *in-vitro* drug release study, for each of the Capecitabine nanoparticle formulation was performed, using Dialysis membrane-60 LA 390-5MT (MWCO 12kDa-14kDa), Hi Media Laboratories Pvt. Ltd (Mumbai, India).At first the dialysis membrane was cut to a desired length

and was cleaned with Milli-Q water followed by rinsing and soaking it for 24 hrs in the respective buffer solution. The freeze dried Capecitabine loaded nanoparticles were redispersed in 2ml of the desired buffer solution. One end of the membrane was mechanically sealed with a dialysis closure and 2ml of the nanoparticle dispersion was poured into the dialysis tubing with the help of a micropipette. The other end of the membrane was sealed and was placed in a 200 ml beaker. 150 ml of the desired buffer solution was poured into the beaker and a magnetic bead was placed in it. The system was stirred at 50 rpm on a magnetic stirrer (2MLH, Remi Equipment Pvt. Ltd., India) at 37 ± 2°C. 1ml of aliquot was taken from the beaker at predetermined time intervals and 1ml of freshly prepared buffer was immediately added to maintain the sink condition. The samples were appropriately diluted with the same buffer solution and were observed under UV-Visible Spectrophotometer at 215 nm.The percentage cumulative drug release was plotted on the Y-axis against time plotted on the X-axis, to understand the release profile of Capecitabine from the nanoparticles.

4.2.6.9. Drug release kinetics study

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from solid-lipid nanoparticles. The kinetic models used were zero order kinetics, first order kinetics, Higuchi model and Korsmeyer-Peppas model.

a) Zero order kinetics

The pharmaceutical dosage forms following zero order kinetics, release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a prolonged pharmacological action. For this model, a graph of the amount of drug released versus time will be linear. The following relation can in a simple way express this model.

$$Q_t = Q_0 + K_0 t$$

Where, $Q_0$is the initial amount of the drug in the solution (most times, Q_0=0),

Q_t is the amount of drug released in the solution at time‘t’,

K_0 is the zero order release constant. [58]

This relation can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs (Varelas *et al.*, 1995), coated forms, osmotic systems etc.

b) First order kinetics

The pharmaceutical dosage forms following this kinetic profile, such as those containing water soluble drugs in porous matrices, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such a way, that the amount of drug released by unit of time diminishes.

This model can be expressed as:

$$logQ_t = logQ_0 - \frac{K_1}{2.303}$$

Where Q_t is the amount of drug released in time‘t’,

$Q_0$is the initial amount of drug in the solution,

K_1 is the first order release constant. [59]

In this way a graph of the decimal logarithm of the released amount of drug versus time will be linear.

c) Higuchi model

Higuchi (1961, 1963) developed several theoretical models to study the release of water soluble and poorly soluble drugs incorporated in semi-solid and/or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media.

In a general way, Higuchi model may be expressed as

$$Q = K_H t^{1/2}$$

Where, Q is the amount of drug released in time't',

$K_H$is the Higuchi dissolution constant. [60]

Higuchi describes drug release as a diffusion process based on Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems (Costa et al., 1996) and matrix tablets with water soluble drugs (Desai et al., 1966;Schwartz et al.,1968).

d) Korsmeyer-Peppas model

Korsmeyer et al.,(1983) developed a simple, semi-empirical model, relating exponentially the drug release to the elapsed time (t),which can be described by the following equation:

$$log\left(\frac{M_t}{M_\infty}\right) = nlogt + logK$$

Where $\left(\frac{M_t}{M_\infty}\right)$ is fraction of drug released at time 't',

n is diffusion exponent indicative of the mechanism of transport of drug through the polymer,

K is kinetic constant (having units of t^{-n}). [61]

When there is a possibility of a burst effect, b, the following equation is used:

$$\frac{M_t}{M_\infty} = at^n + b$$

Where, 'a' is a constant incorporating structural and geometrical characteristic of the drug dosage form. [62]

Peppas (1985) used this 'n' value in order to characterize different release mechanisms, concluding for values for a slab, of n=0.5 for Fick diffusion and higher values of 'n', between 0.5 and 1.0, or n=1.0 for mass transfer following a non-Fickian model (Table 4.2; Table 4.3).

This model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved. This model has also been used to describe the drug release from several different pharmaceutical modified release dosage forms (Lin and Yang, 1989; Sangalli et al., 1994; Kim and Fassihi, 1997).

Table 4.2: Interpretation of diffusional mechanism

Release exponent 'n'	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<n<1.0	Anomalous transport (non-Fickian diffusion)	t^{n-1}
1.0	Case-II transport	Zero order release
Higher than 1.0	Super case--II transport	t^{n-1}

Table 4.3: Korsmeyer- Peppas diffusion exponent and mechanism of diffusional release from various swellable controlled release systems. [61]

Drug release mechanism	
Spherical sample	Type of diffusion
≤0.5	Fickian diffusion
0.5<n<1.0	Non-Fickian diffusion

CHAPTER 5:
TABLES AND GRAPHS

5. Tables and Graphs

Table 5.1.Physical interactions of Drug and Excipients.

Materials	Initial		Final	
	Colour	State	Colour	State
Drug	White	Solid ,free flowing, powdery mass	No change	Solid, granular mass
Drug+Chitosan	Off-White	Solid ,free flowing, powdery mass	No change	No change
Drug+Sodium tripolyphosphate	White	Solid ,free flowing, powdery mass	No change	No change
Drug+Acetone	Drug dissolves in Acetone to produce a colourless solution	Liquid	White	Acetone evaporated leaving behind a very minute amount of solid mass of the drug as residue
Drug+Gelatin	Whitish yellow	Solid ,free flowing, powdery mass	Yellow	Solid, rigid mass
Drug+Glutaraldehyde	Undissolved drug (White) dispersed in light yellow coloured glutaraldehyde solution	Colloidal solution of dispersed drug in glutaraldehyde	Golden yellow	Liquid solution of dissolved drug in glutaraldehyde
Drug+HPMC	White	Solid ,free flowing, powdery mass	No change	No change
Drug+Ethanol	Drug dissolves in Ethanol to produce a colourless solution	Liquid	____	Ethanolic solution evaporated completely leaving behind no residue
Drug+ Carbopol (940)	White	Solid ,free flowing, powdery mass	No change	Solid, rigid mass
Drug+ CAP	White	Solid, free flowing, powdery mass	No change	Solid ,free flowing, powdery mass

Table 5.2. Particle size, PDI and Zeta Potential of Capecitabine nanoparticles.

Formulations	Average particle size(nm)	PDI	Zeta Potential(mV)
CN-1	340.6	0.382	14.7
CN-2	288.3	0.311	20.3
CN-3	263.9	0.307	21.89
CN-4	166.5	0.346	37.4
CN-5	188.3	0.354	24.6
CN-6	218.4	0.322	25.75
CN-7	331.7	0.579	19.85
CN-8	468.3	0.471	24.67
CN-9	726.4	0.792	17.56

Table 5.3. Entrapment Efficiency and Percentage Yield of Capecitabine loaded nanoparticles.

Formulations	Entrapment efficiency (%)	Percentage yield (%)
CN-1	30.12	83.49
CN-2	45.89	76.81
CN-3	63.59	81.26
CN-4	65.34	90.32
CN-5	55.28	92.56
CN-6	51.03	87.11
CN-7	48.53	81.32
CN-8	39.72	78.35
CN-9	34.36	80.57

Table 5.4. Cumulative Percentage Release of different formulations in pH 1.2.

Time (hrs)	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6	CN-7	CN-8	CN-9
0	0	0	0	0	0	0	0	0	0
1.5	64.998	62.862	51.657	53.655	54.323	59.876	52.567	49.889	48.243
2	67.138	65.399	63.896	56.426	57.876	57.564	56.987	54.879	50.001
2.5	68.999	66.895	64.123	58.896	58.994	54.456	57.976	58.991	58.112
3	71.106	69.916	65.634	61.668	58.208	55.779	58.432	62.865	58.501
4	72.365	71.343	67.893	62.838	59.998	57.791	59.543	64.356	59.431
5	74.436	72.987	69.345	69.577	61.767	59.987	61.324	65.435	60.090
6	77.809	74.897	71.748	72.800	63.678	61.985	64.234	65.785	61.765
12	79.841	78.123	72.149	74.998	64.987	63.875	66.876	66.985	63.985
24	80.797	79.084	75.647	79.675	65.023	64.987	67.894	68.903	65.679
25	80.857	81.674	76.806	80.551	66.866	65.004	69.876	69.234	68.755

Table 5.5. Cumulative Percentage Release of different formulations in pH 6.8.

Time (hrs)	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6	CN-7	CN-8	CN-9
0	0	0	0	0	0	0	0	0	0
1.5	54.844	52.862	41.647	43.332	52.453	48.865	52.567	49.889	47.243
2	57.138	58.699	53.092	52.466	55.866	51.564	56.916	54.879	49.674
2.5	58.999	63.795	55.131	58.896	57.994	53.457	57.976	58.990	55.684
3	59.106	65.906	56.664	65.668	58.258	55.789	58.432	62.865	58.843
4	62.365	68.323	57.893	69.838	58.998	57.987	59.543	64.356	60.431
5	67.496	72.687	59.345	76.577	61.067	59.980	61.124	65.435	63.466
6	67.001	74.807	67.748	79.897	62.608	61.565	64.466	65.785	61.765
12	69.140	77.132	72.143	82.456	64.087	63.505	66.8765	66.984	66.875
24	70.791	78.086	74.647	85.675	65.003	64.711	67.843	68.900	69.789
25	72.412	81.674	75.806	85.850	64.896	65.791	70.873	69.873	78.645

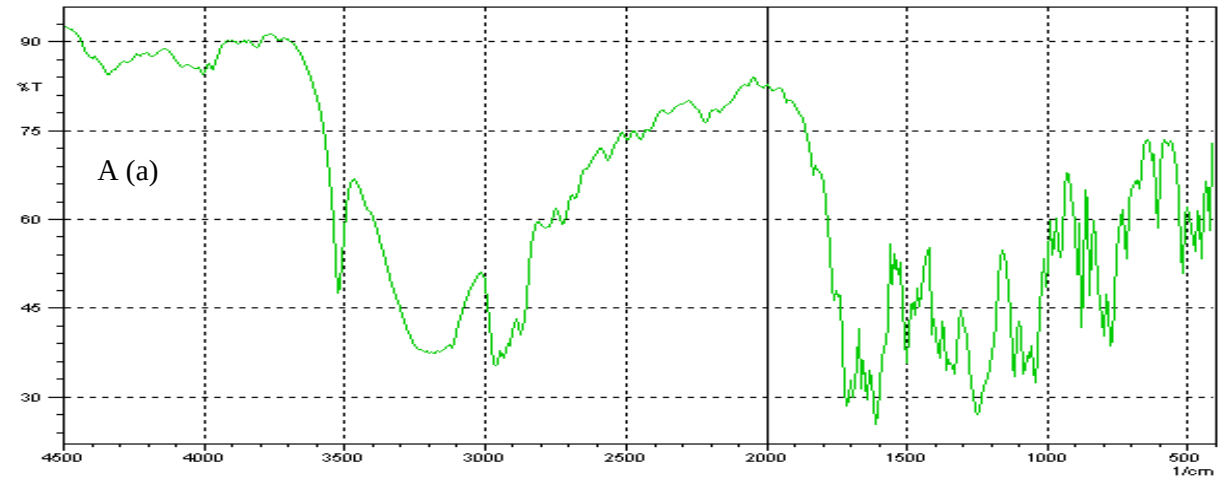
Table 5.6. Cumulative Percentage Release of different formulations in pH 7.4.

Time (hrs)	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6	CN-7	CN-8	CN-9
0	0	0	0	0	0	0	0	0	0
1.5	44.998	46.466	9.823	56.554	48.108	58.163	59.983	28.001	45.491
2	47.438	50.898	15.892	60.466	54.332	62.945	65.221	36.006	49.093
2.5	47.999	53.123	19.565	65.896	56.589	64.345	67.544	38.234	52.966
3	50.106	56.234	26.978	68.564	59.661	66.455	74.861	42.567	57.147
4	55.335	60.084	33.643	70.838	62.345	70.845	78.967	48.345	61.834
5	56.436	68.989	37.698	72.577	65.234	72.124	81.453	58.546	66.512
6	57.809	71.499	43.763	75.098	67.419	75.986	83.322	60.424	71.779
12	59.847	80.1204	58.009	83.567	71.441	80.009	90.346	70.146	77.567
24	70.791	90.981	67.978	90.811	73.458	82.545	94.323	77.127	85.561
25	75.857	92.865	68.565	90.854	74.441	84.997	95.321	80.806	88.153

Table 5.7.*In vitro* release kinetic parameters of various model equations.

Formulation code	Zero-order		First-order		Higuchi plot		Korsmeyer-Peppas plot	
	k_0	R^2	k_1	R^2	k_H	R^2	n	R^2
CN-1	1.082	0.917	0.027	0.942	38.20	0.952	0.257	0.960
CN-2	1.731	0.872	0.069	0.959	36.91	0.947	0.242	0.976
CN-3	2.163	0.836	0.041	0.922	0.011	0.927	0.549	0.937
CN-4	1.223	0.853	0.059	0.969	51.91	0.934	0.160	0.971
CN-5	0.805	0.685	0.046	0.861	48.72	0.804	0.120	0.941
CN-6	0.868	0.764	0.032	0.851	55.76	0.871	0.123	0.947
CN-7	1.181	0.732	0.082	0.937	57.96	0.842	0.154	0.914
CN-8	1.817	0.796	0.048	0.905	21.64	0.894	0.344	0.926
CN-9	1.514	0.822	0.055	0.936	38.87	0.913	0.226	0.952

R is the correlation coefficient, k_0 is the zero order release rate constant, k_1 is the first order release rate constant, k_H is the Higuchi constant and 'n' is the release exponent of Korsmeyer-Peppas model.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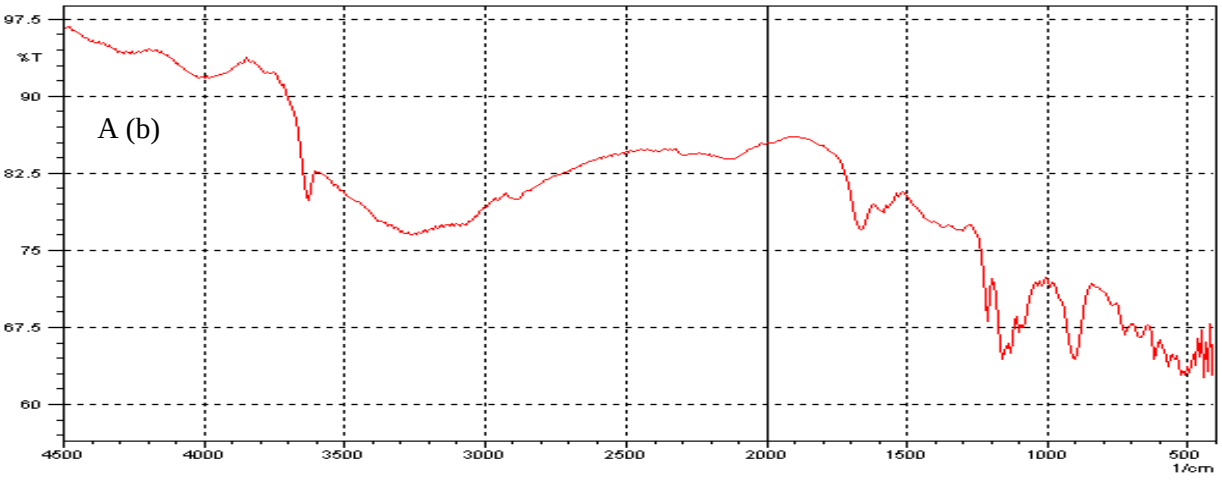
45
30
4500
4000
3500
3000
2500
2000
1500
1000
500
1/cm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3000
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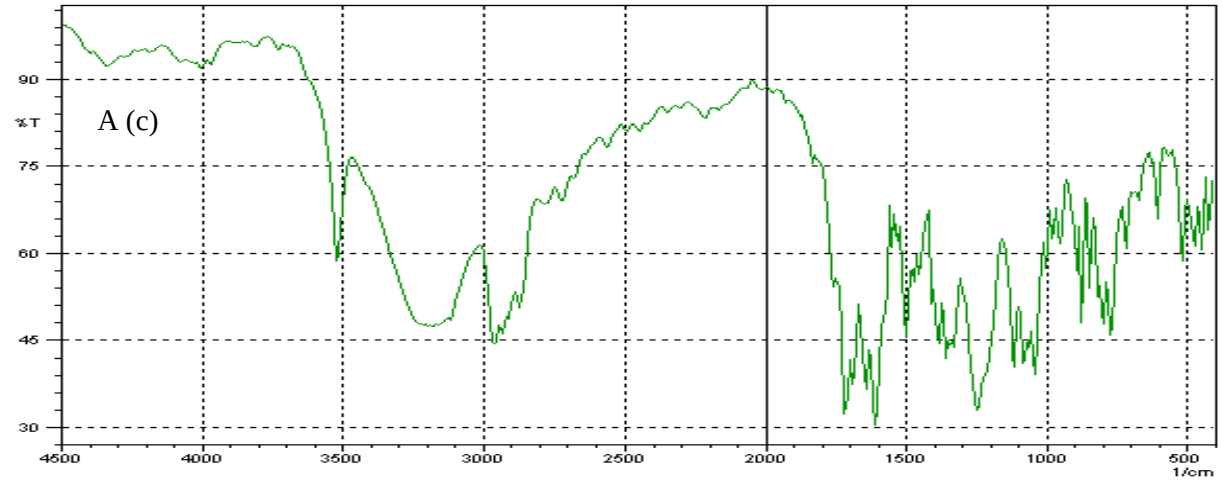
500
1/cm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%T
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3500
3000
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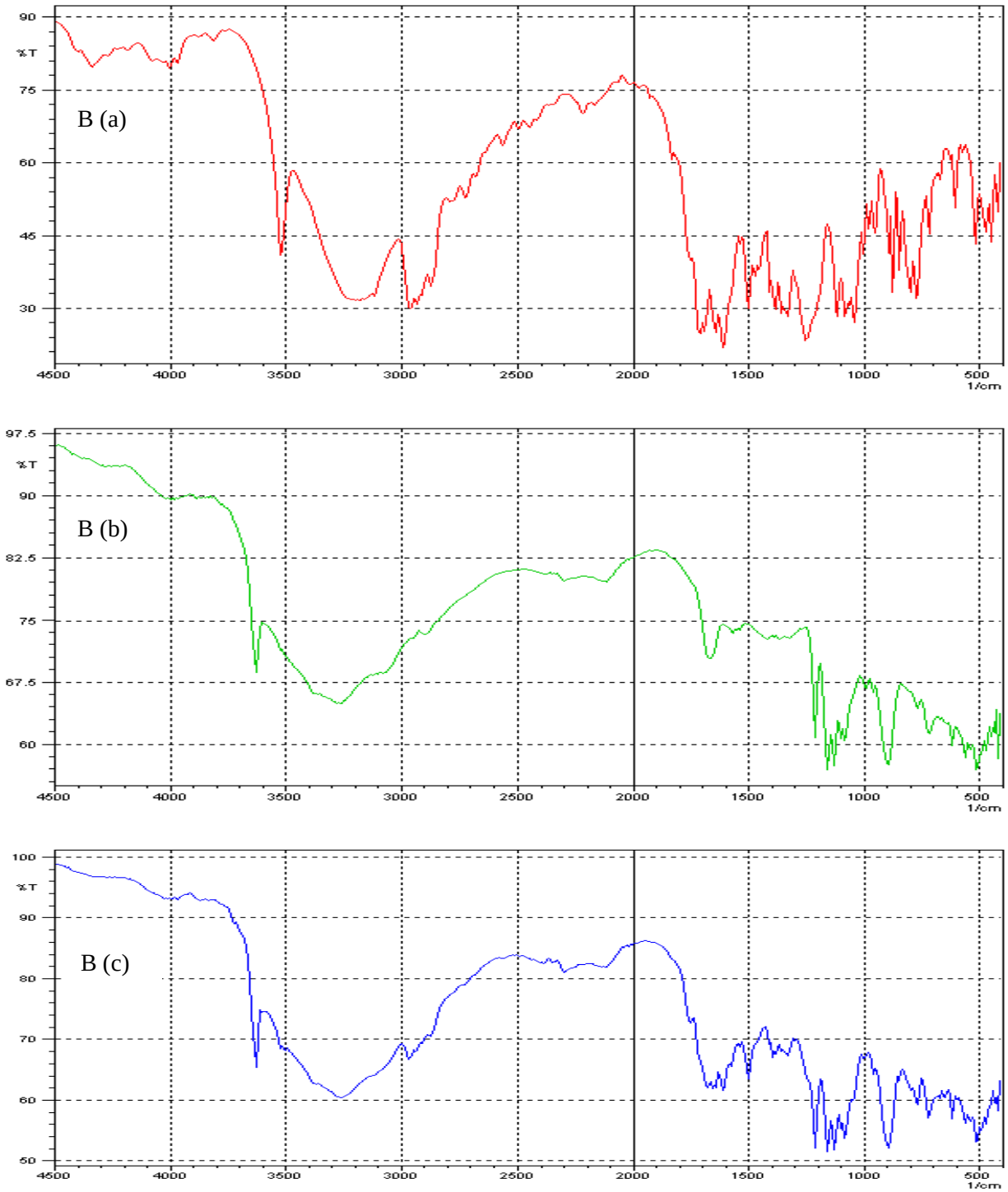

Fig 5.1.Drug - Excipient compatibility study (A).Initial FTIR spectra of (a).Capecitabine. (b).LMW Chitosan+STPP (Excipient blend). (c).Capecitabine+excipients. (B).Final FTIR spectra of (a).Capecitabine. (b).LMW Chitosan+STPP (Excipient blend). (c).Capecitabine+excipients.

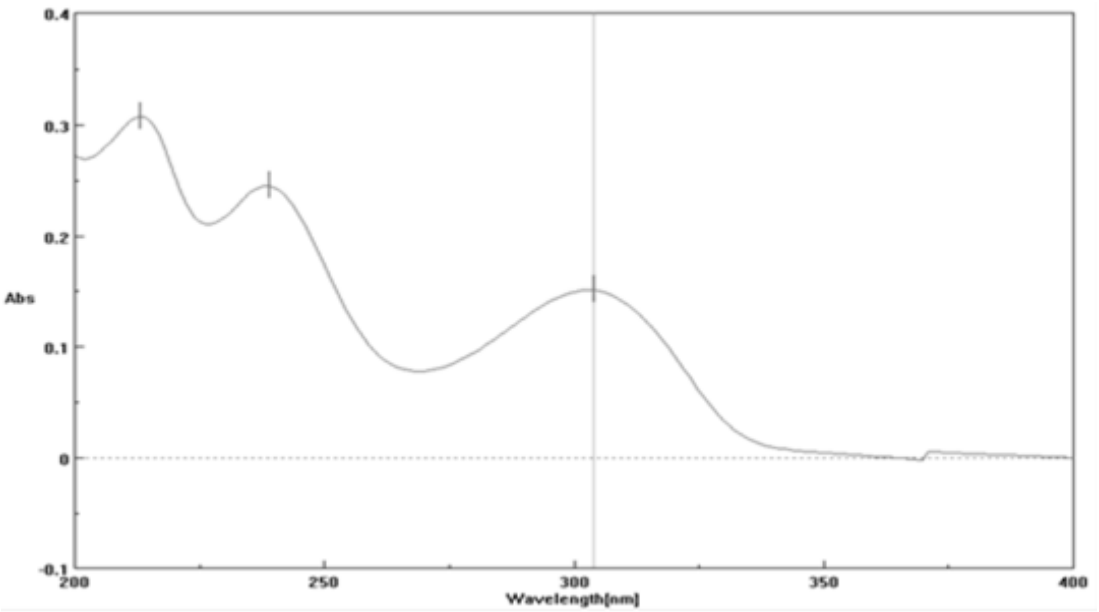

Fig 5.2.Determination of λ_{max} of Capecitabine in Milli-Q water.

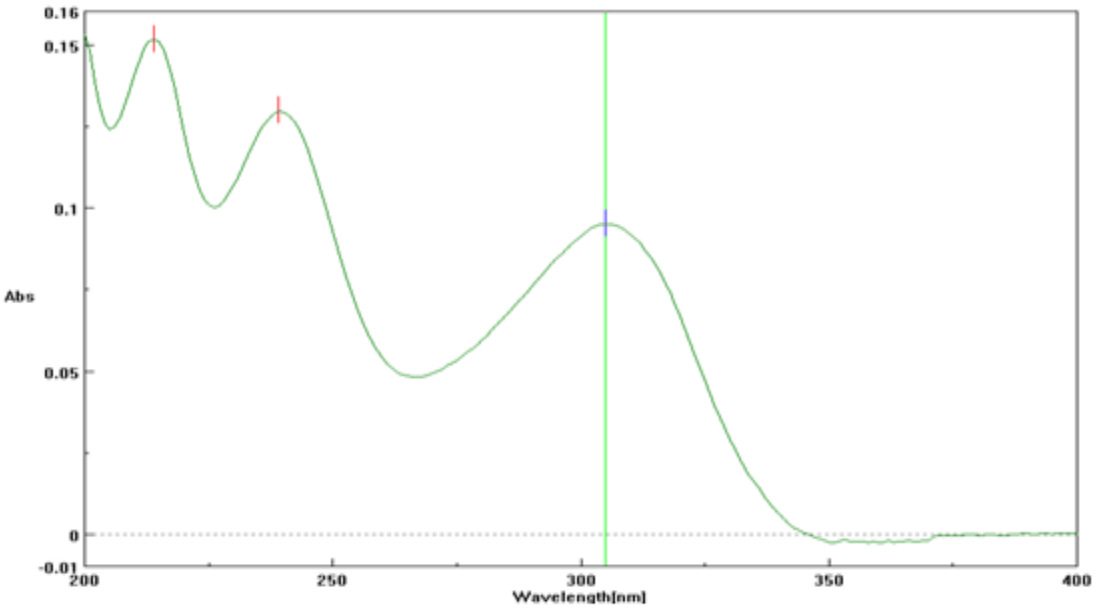

Fig 5.3.Determination of λ_{max} of Capecitabine in 0.1MHCl (pH 1.2).

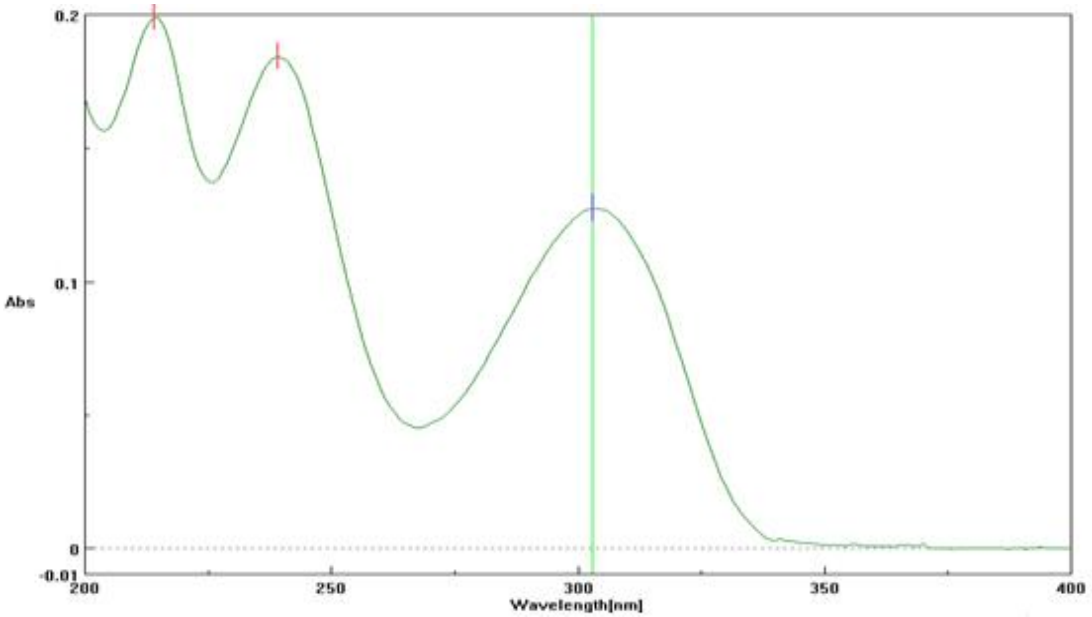

Fig 5.4.Determination of λ_{max} of Capecitabine in pH 6.8.

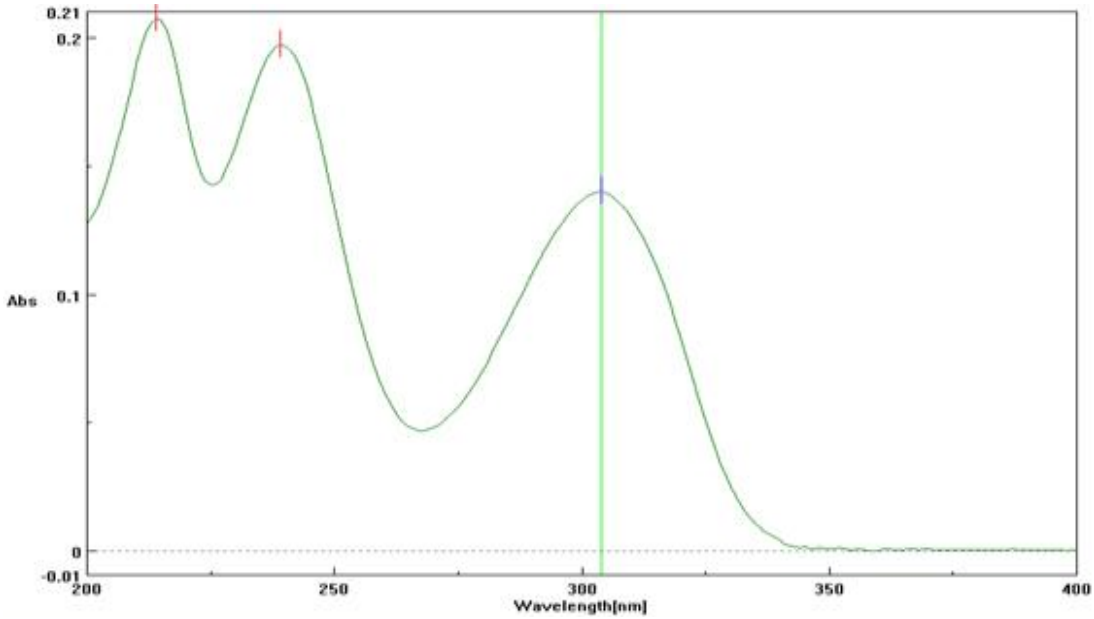

Fig 5.5.Determination of λ_{max} of Capecitabine in pH 7.4.

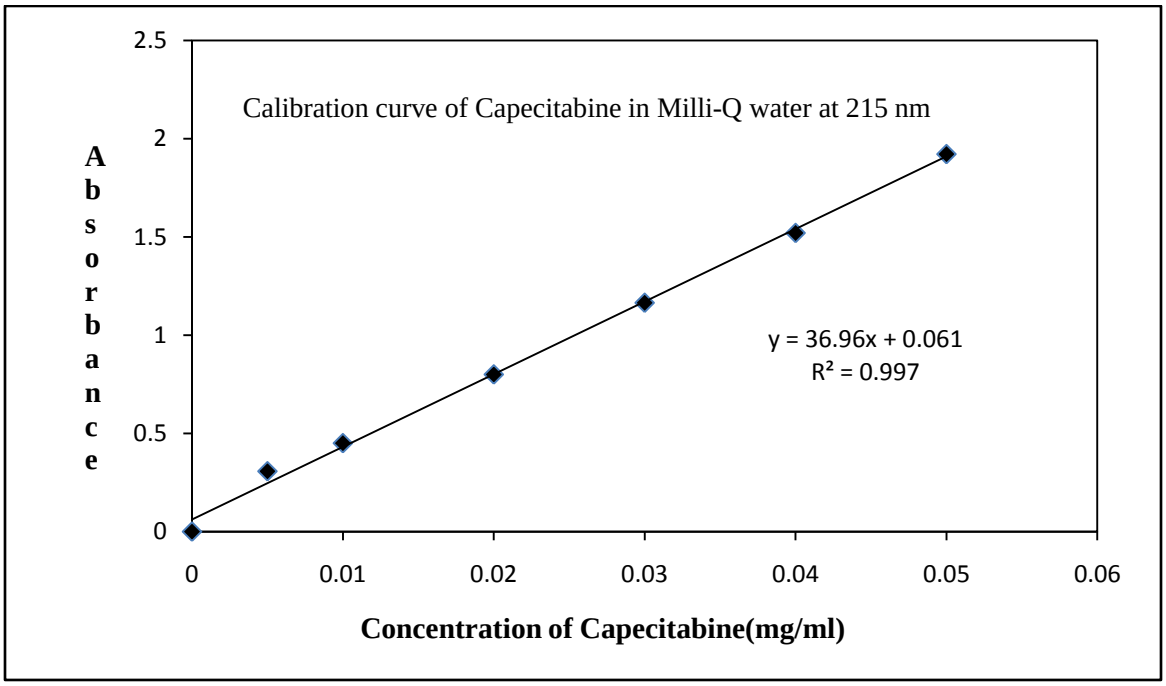

Fig 5.6.Calibration curve of Capecitabine in Milli-Q water at 215 nm.

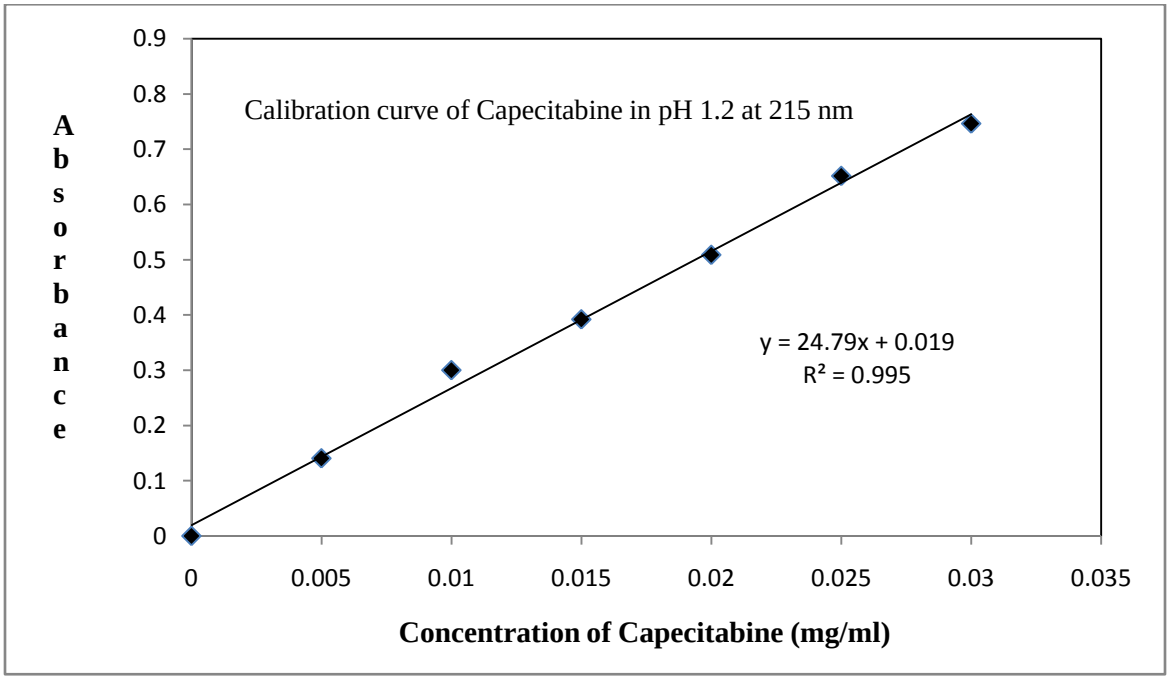

Fig 5.7.Calibration curve of Capecitabine in 0.1M HCl pH 1.2 at 215 nm.

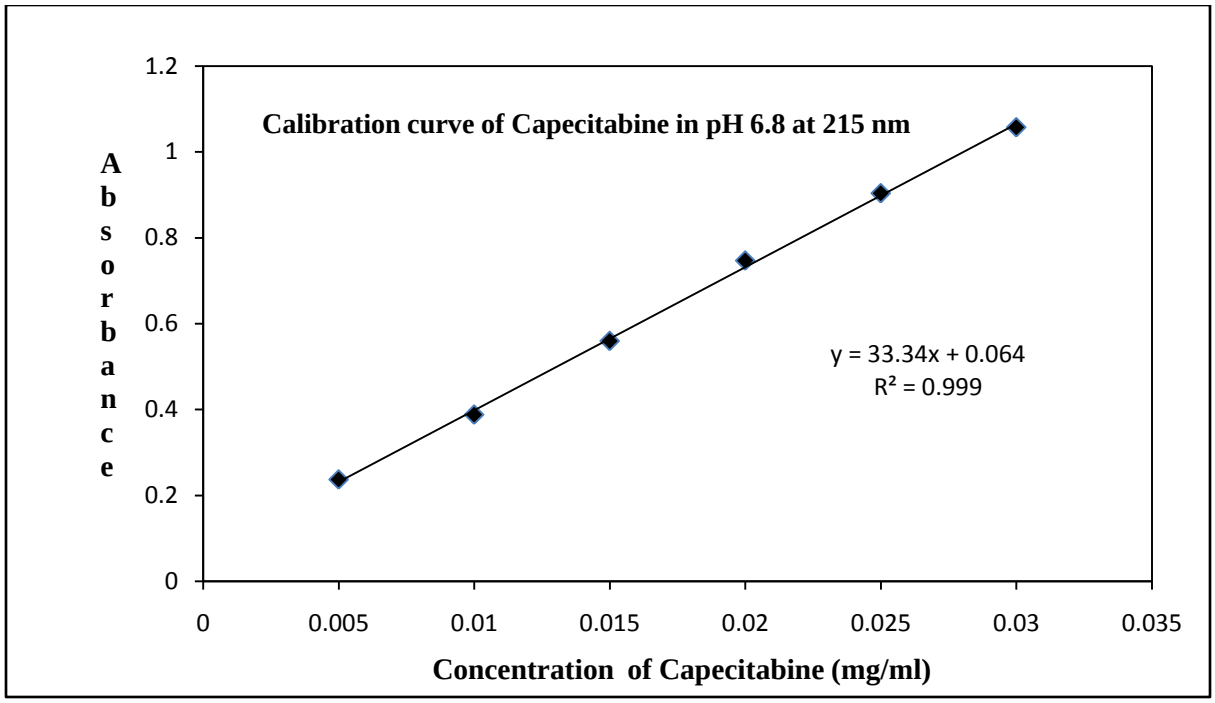

Fig 5.8.Calibration curve of Capecitabine in pH 6.8 at 215 nm.

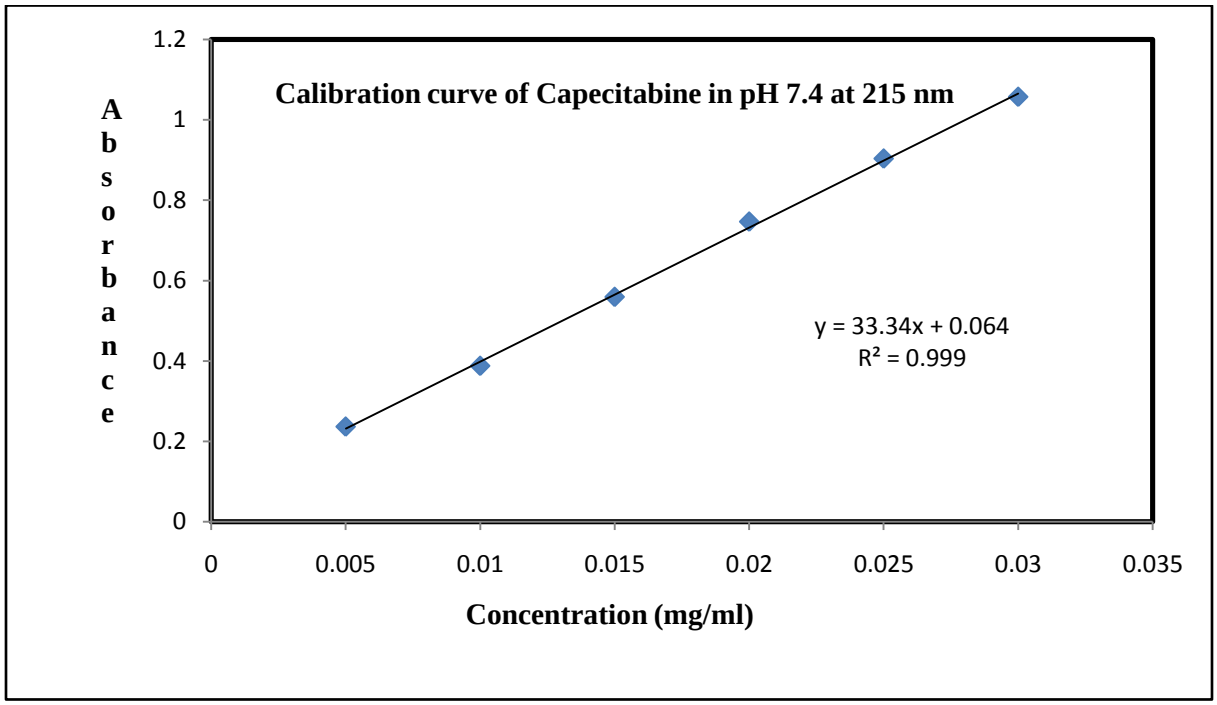

Fig 5.9.Calibration curve of Capecitabine in pH 7.4 at 215 nm.

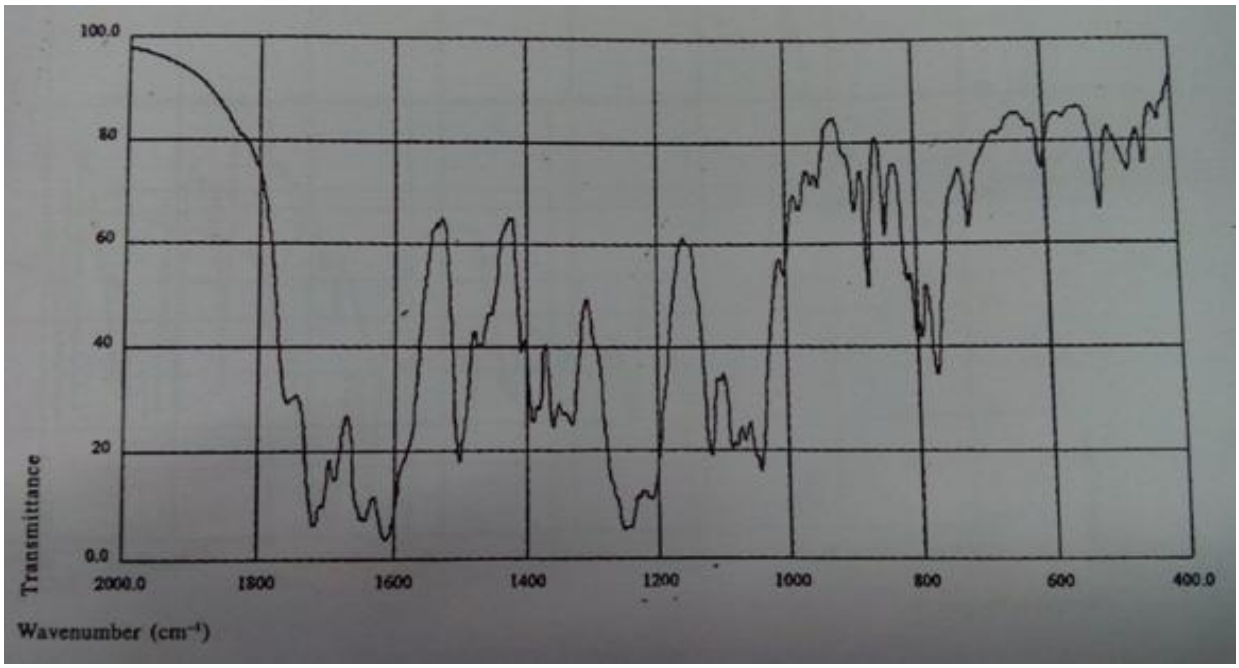

Fig 5.10.FT-IR spectrum of Capecitabine (reference in Indian Pharmacopoeia 2014).

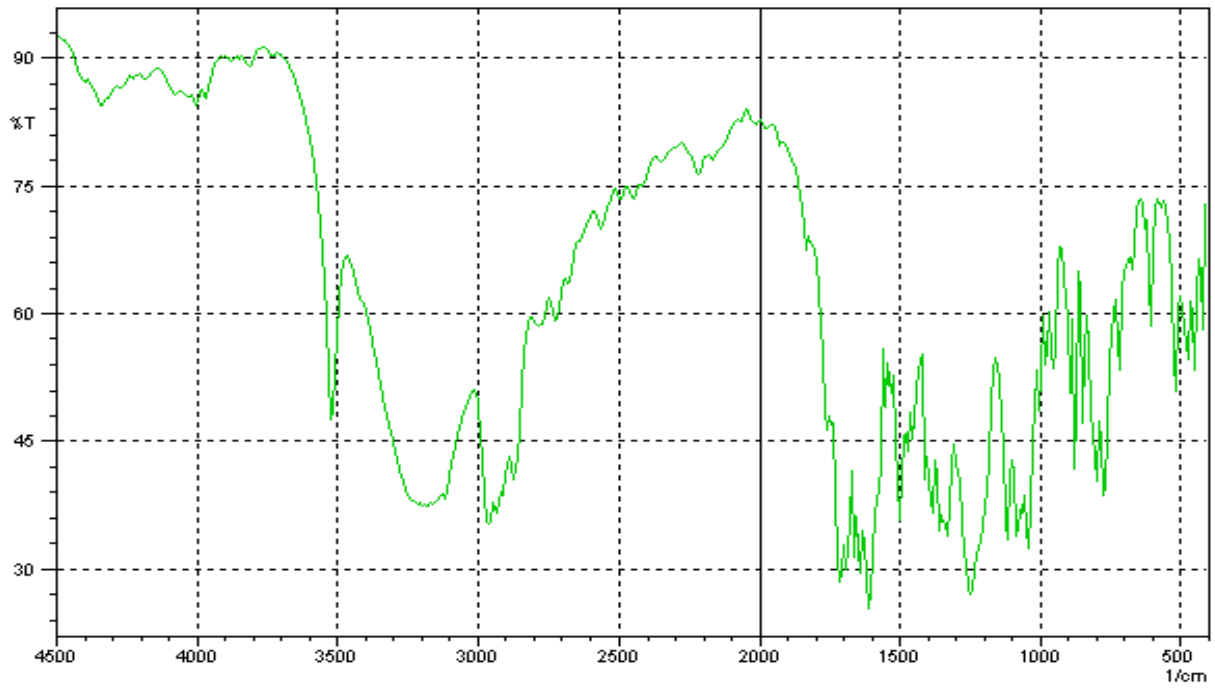

Fig 5.11. FT-IR spectrum of Capecitabine.

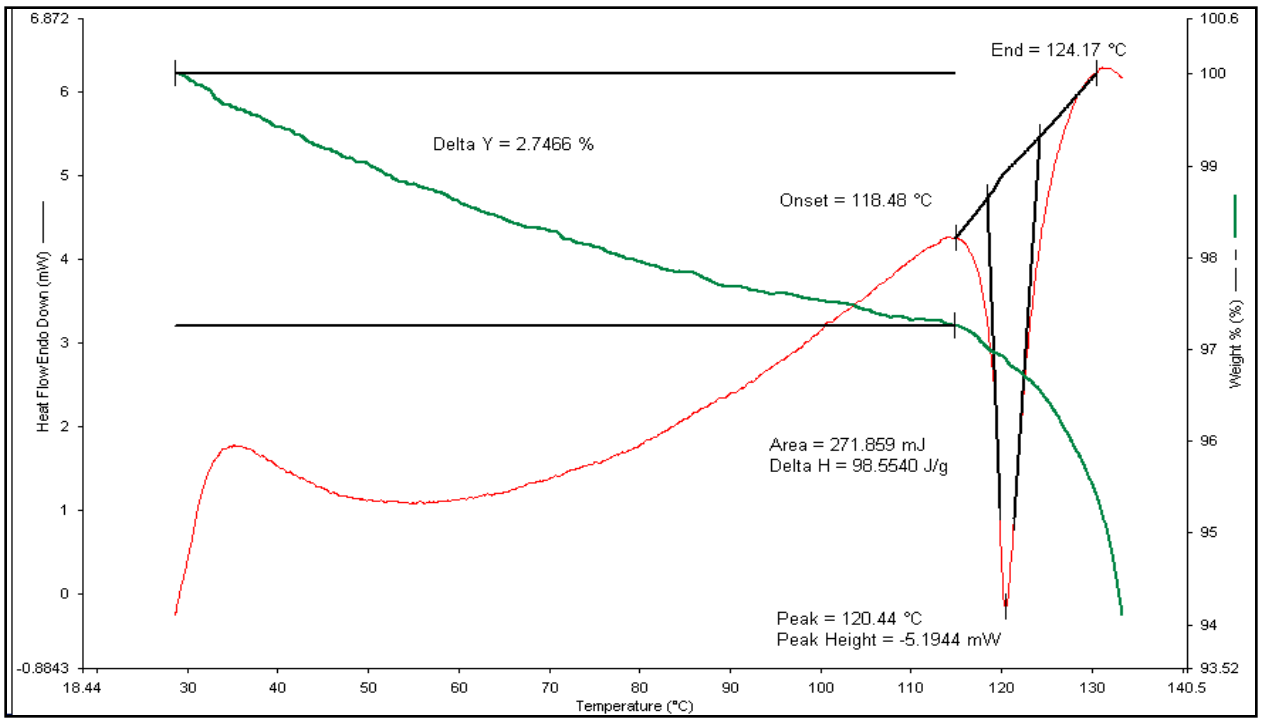

Fig 5.12. DSC of pure Capecitabine.

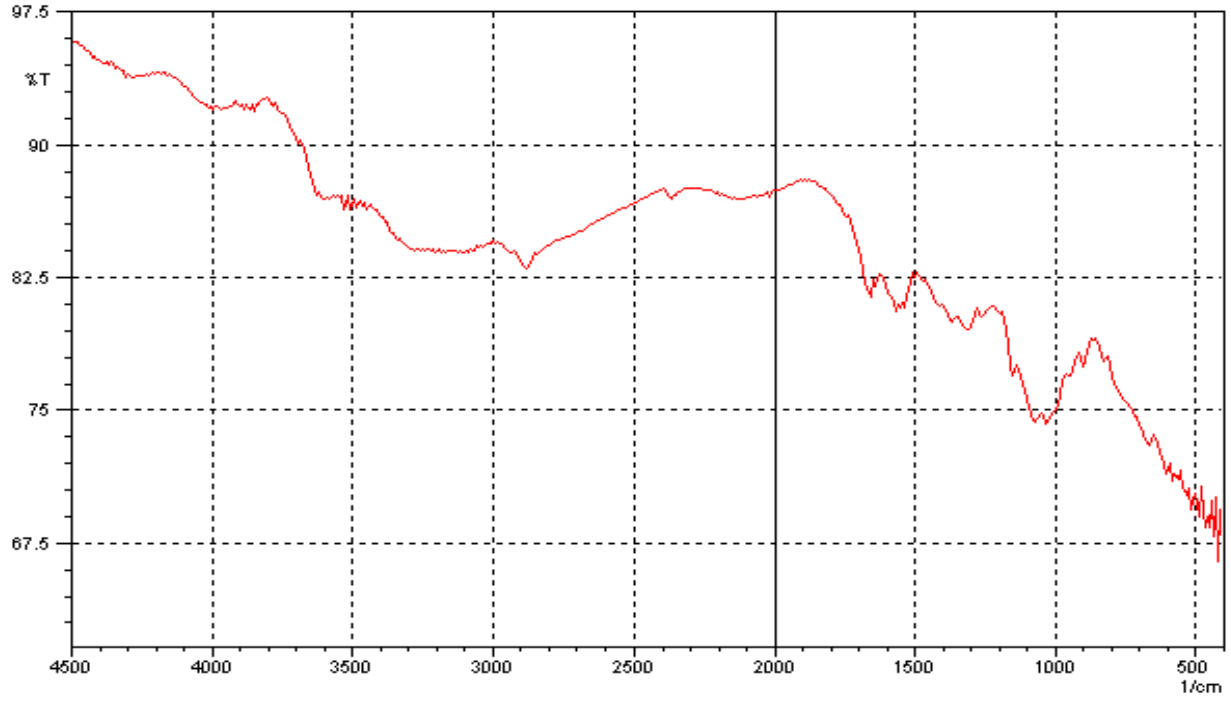

Fig 5.13.FT-IR spectrum of Chitosan.

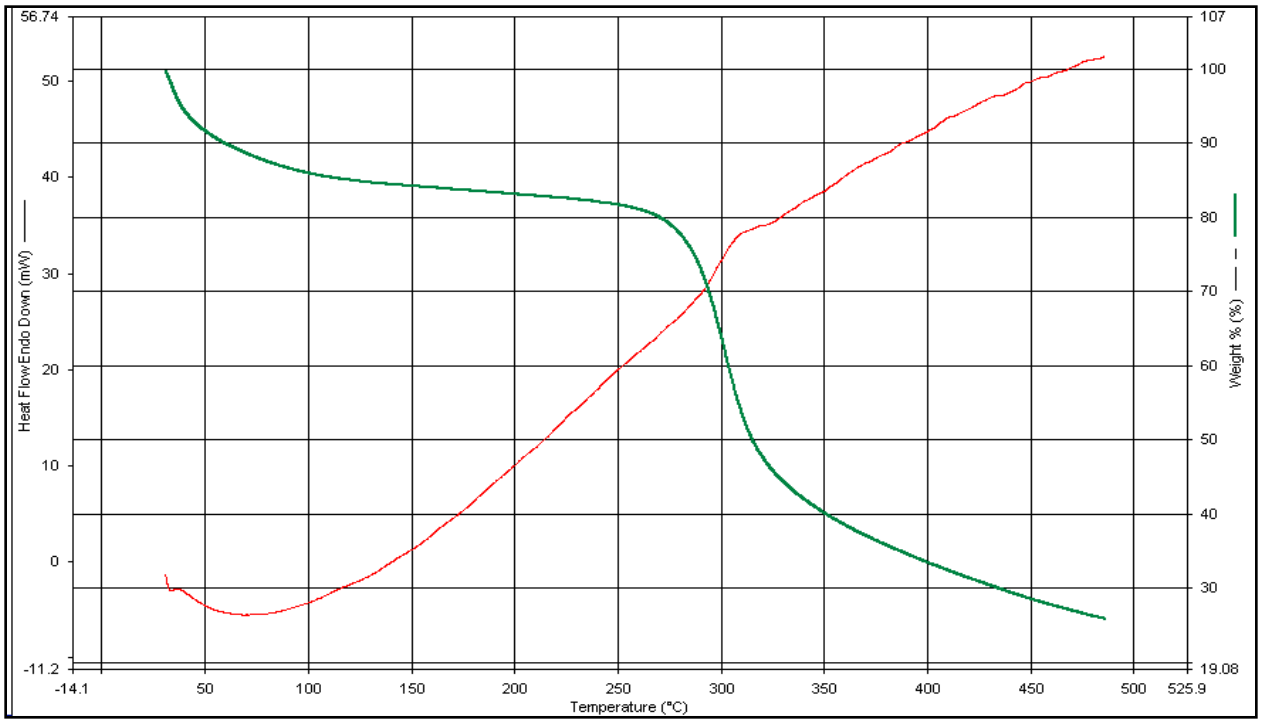

Fig 5.14. DSC of Chitosan.

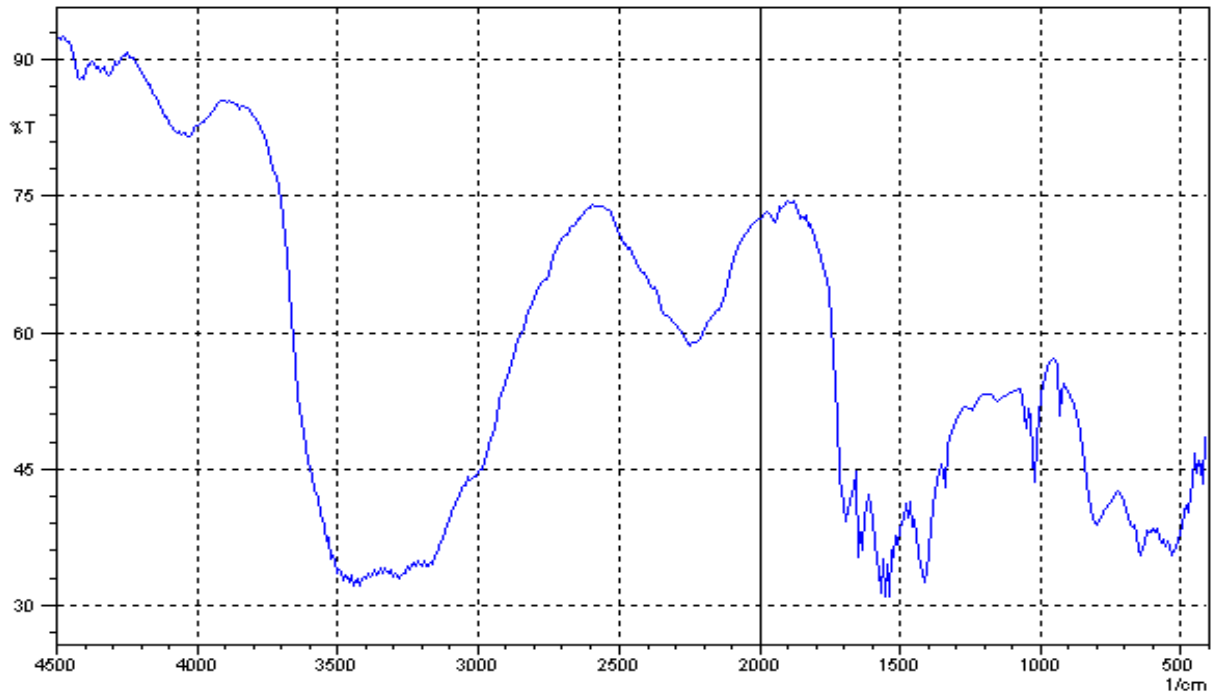

Fig 5.15. FT-IR spectrum of blank chitosan-tripolyphosphate nanoparticles.

Fig 5.16.FT-IR spectrum of Capecitabine loaded nanoparticles (CN-4).

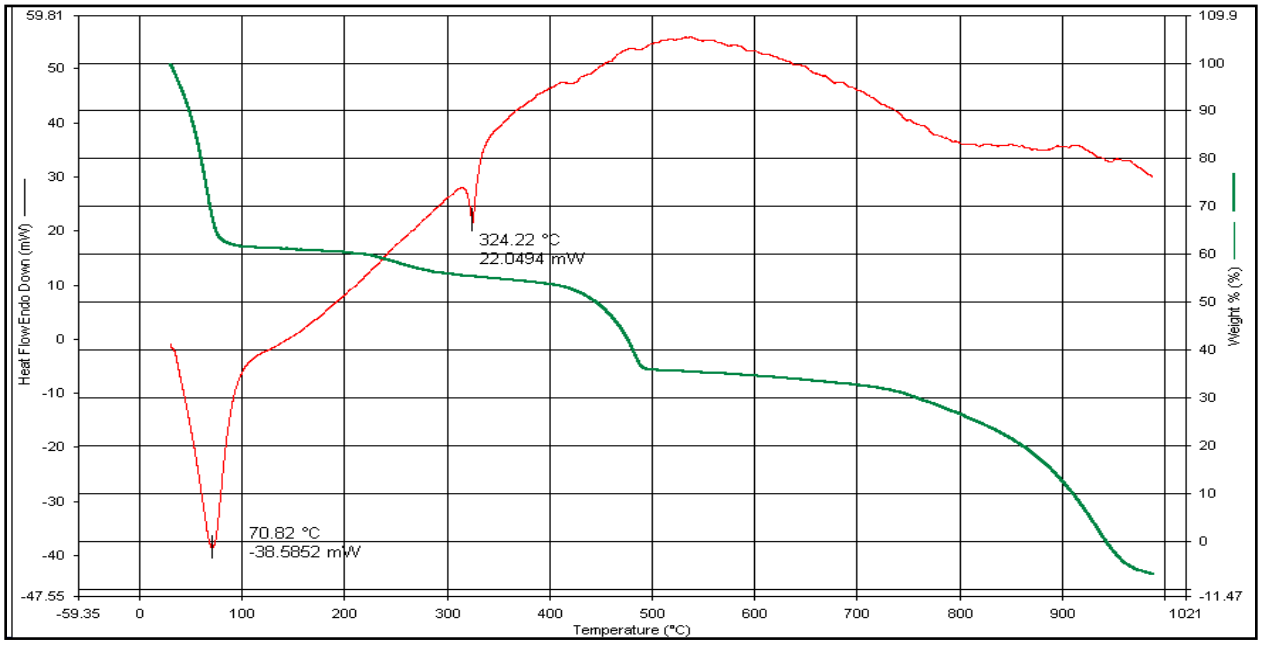

Fig 5.17.DSC of blank chitosan-tripolyphosphate nanoparticles.

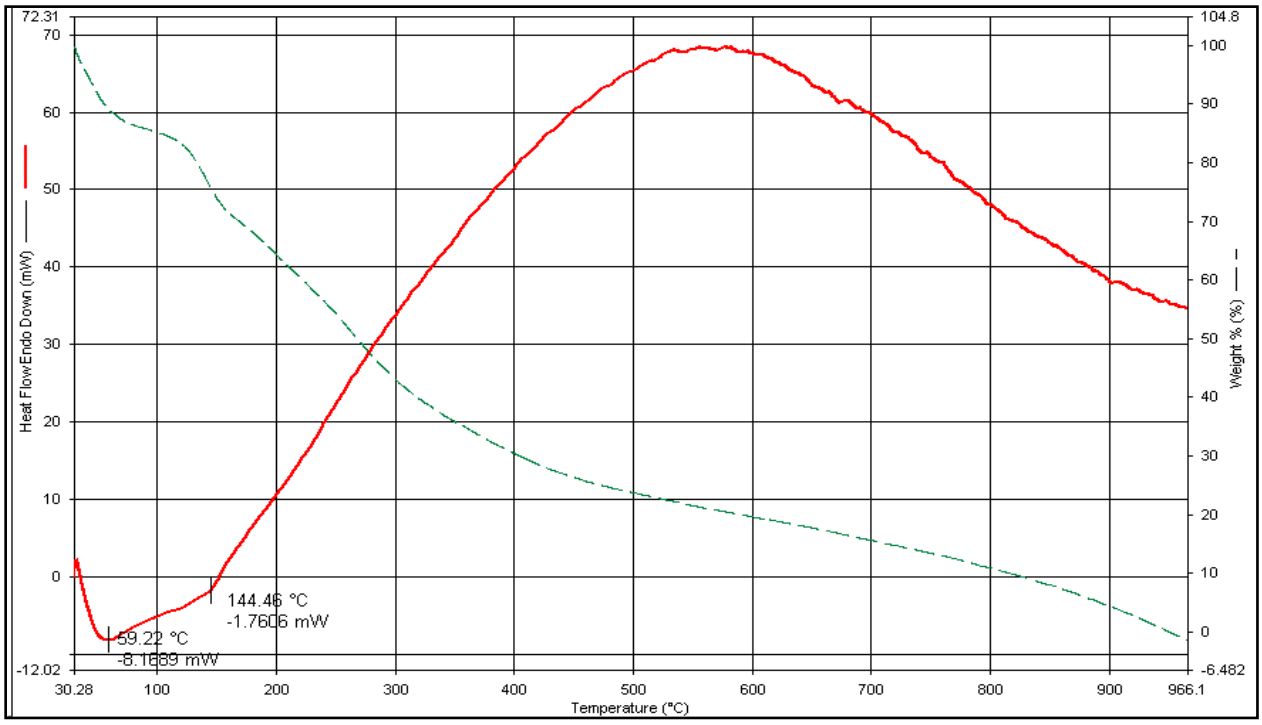

Fig 5.18.DSC of Capecitabine loaded nanoparticles (CN-4).

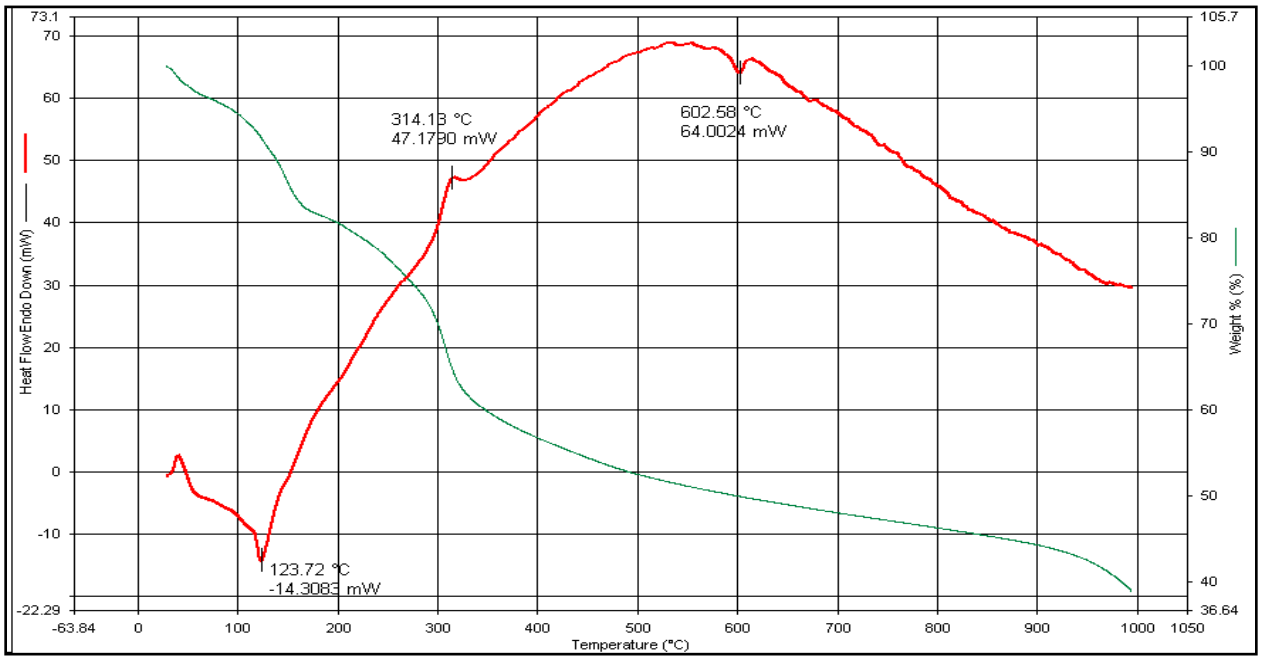

Fig 5.19. DSC of Capecitabine with the excipient blend.

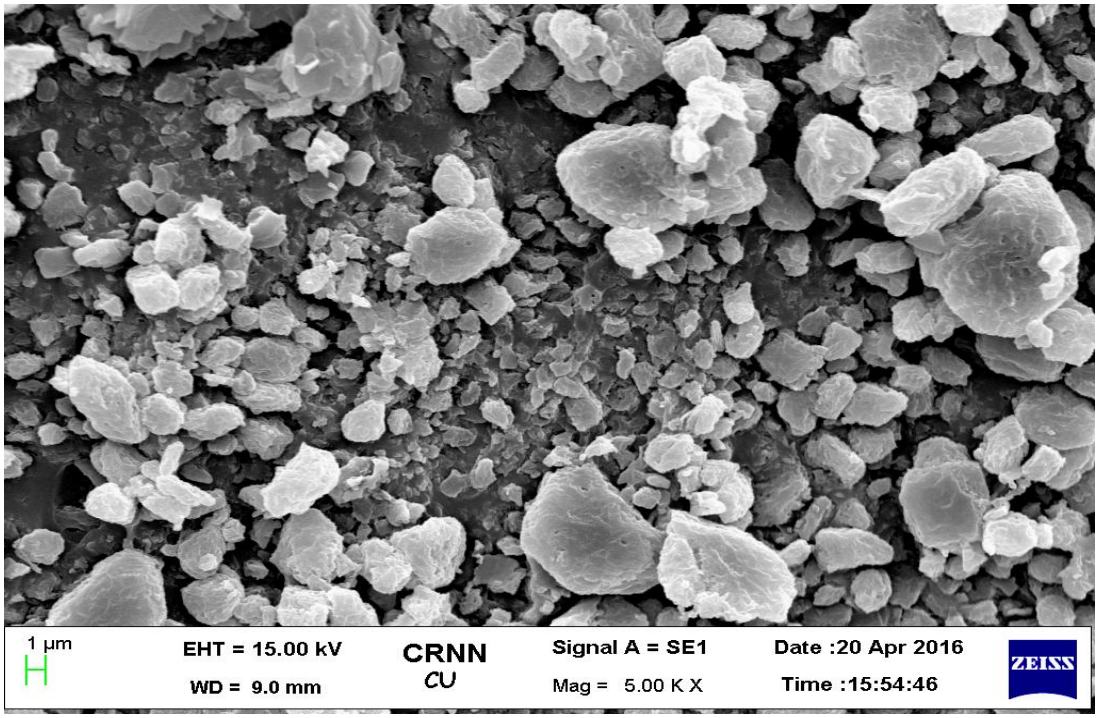

Fig 5.20. SEM of Capecitabine loaded nanoparticles (CN-4).

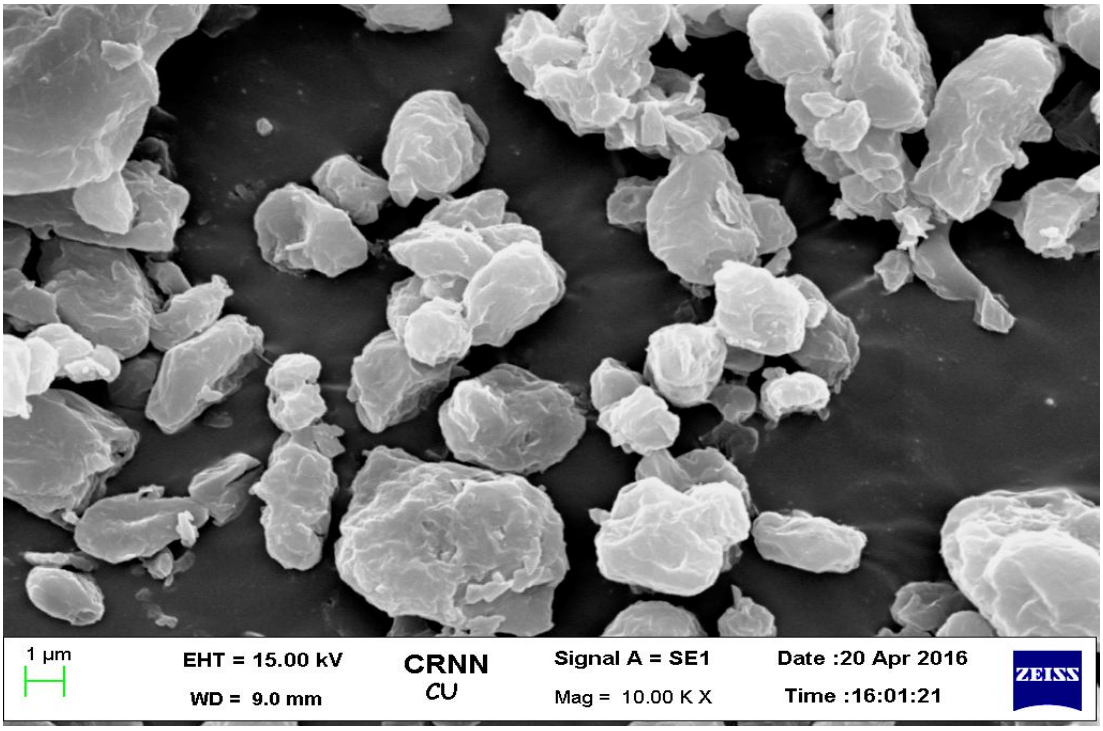

Fig 5.21.SEM of Capecitabine loaded nanoparticles (CN-4).

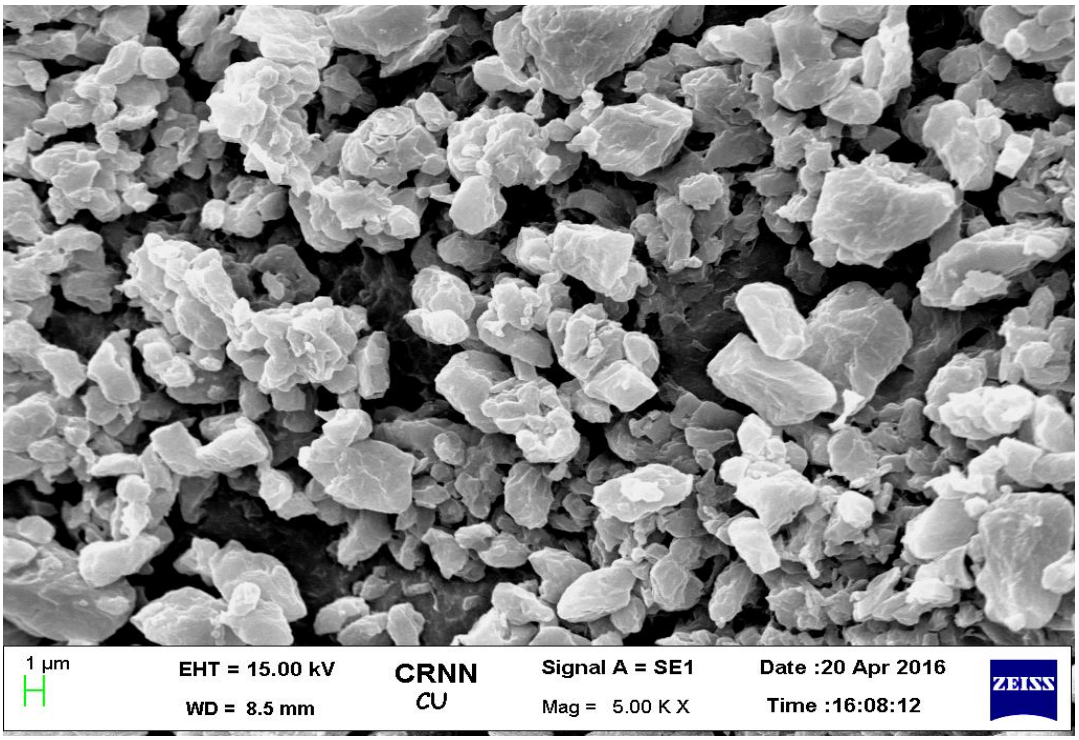

Fig 5.22.SEM of Capecitabine loaded nanoparticles (CN-5).

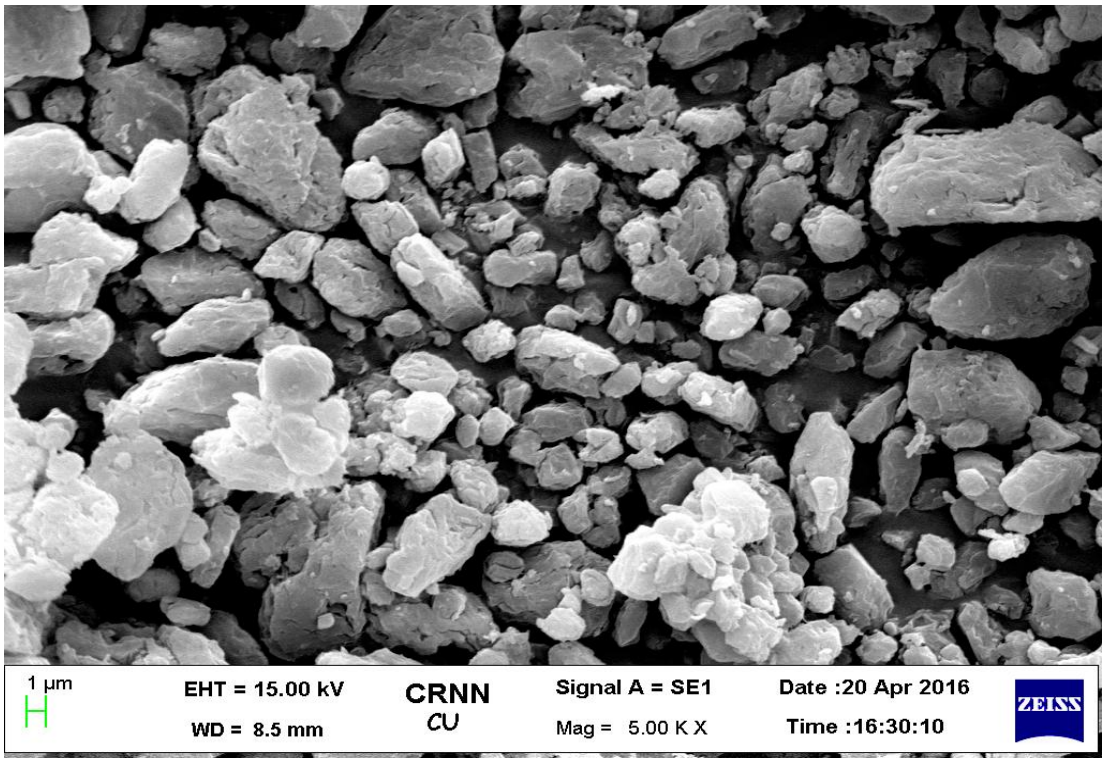

Fig 5.23.SEM of Capecitabine loaded nanoparticles (CN-5).

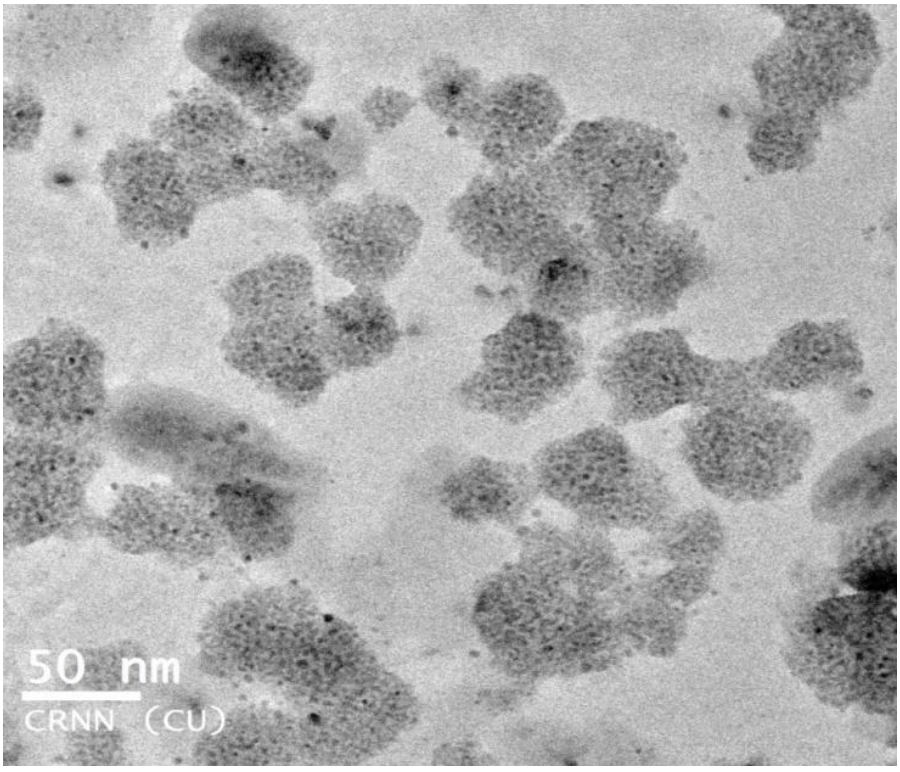

Fig 5.24.TEM of Capecitabine loaded nanoparticles (CN-4).

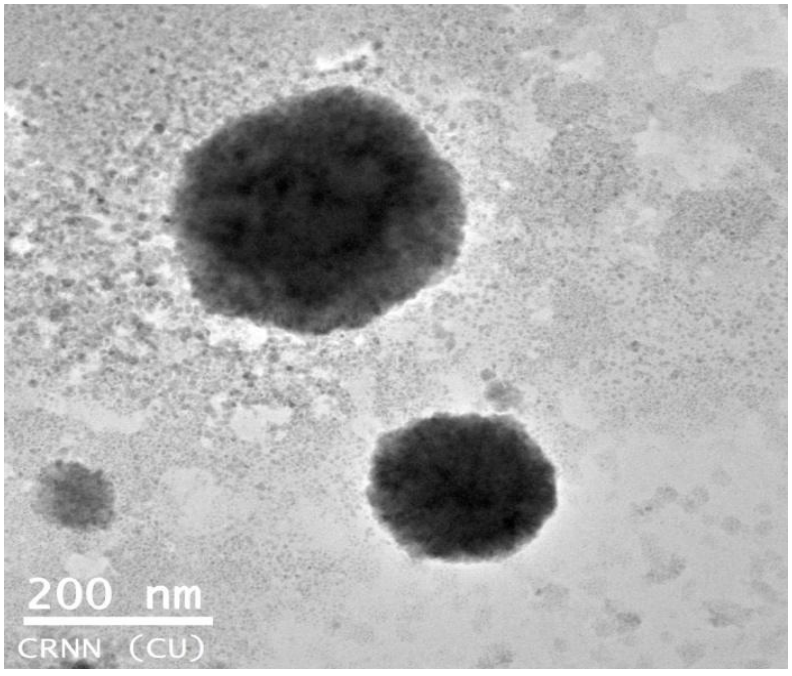

Fig 5.25.TEM of Capecitabine loaded nanoparticles (CN-4).

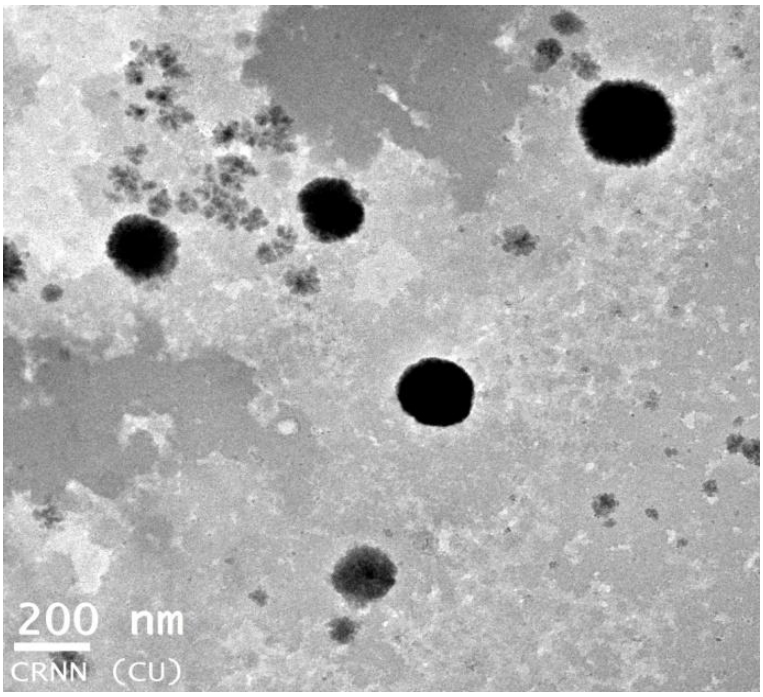

Fig 5.26.TEM of Capecitabine loaded nanoparticles (CN-5).

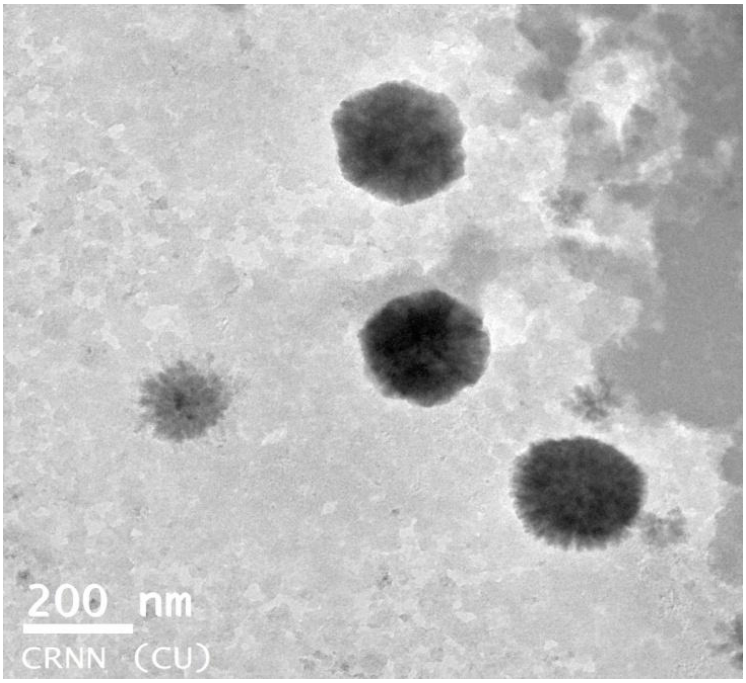

Fig 5.27.TEM of Capecitabine loaded nanoparticles (CN-5).

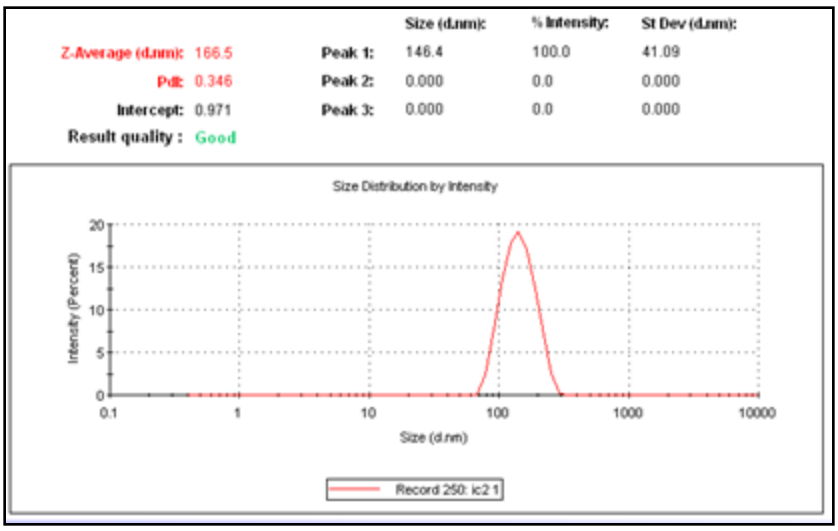

Fig 5.28.Particle size distribution of CN-4.

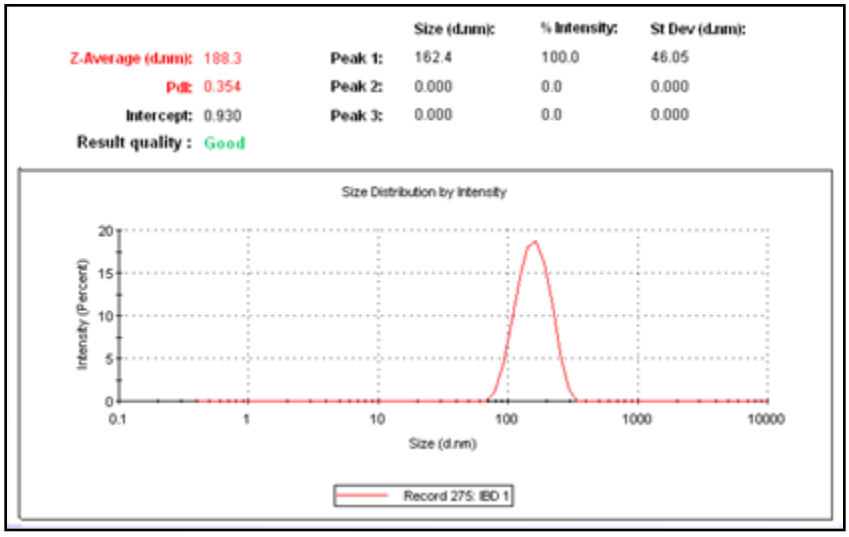

Fig 5.29.Particle size distribution of CN-5.

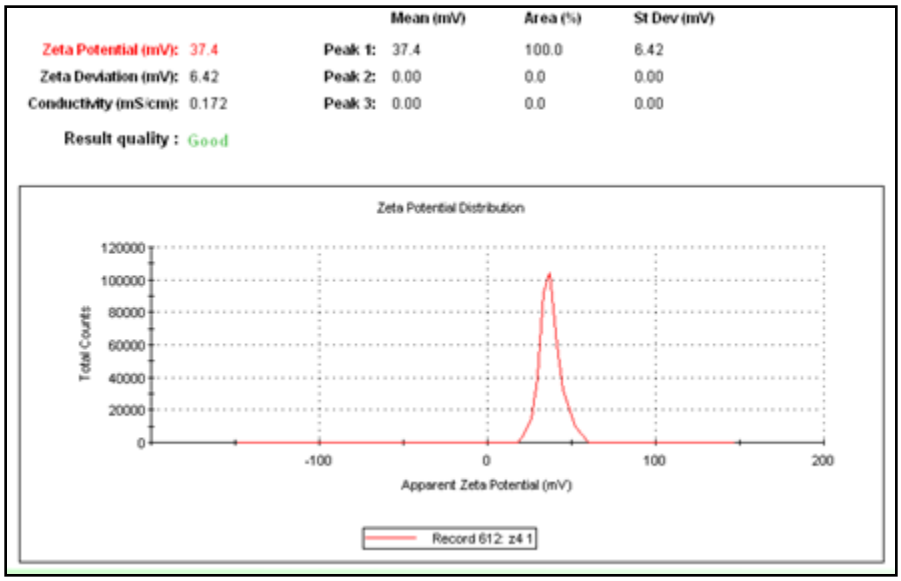

Fig 5.30.Zeta Potential of CN-4.

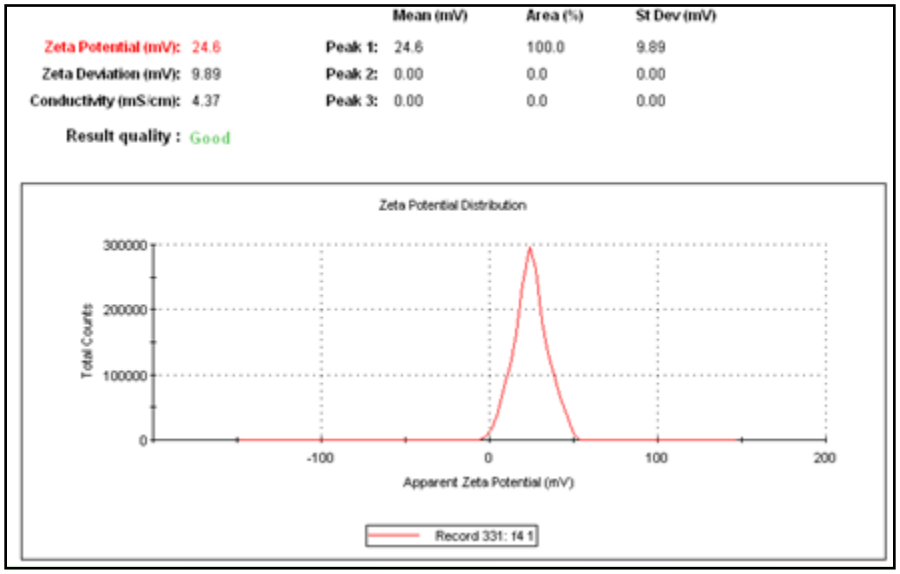

Fig 5.31.Zeta Potential of CN-5.

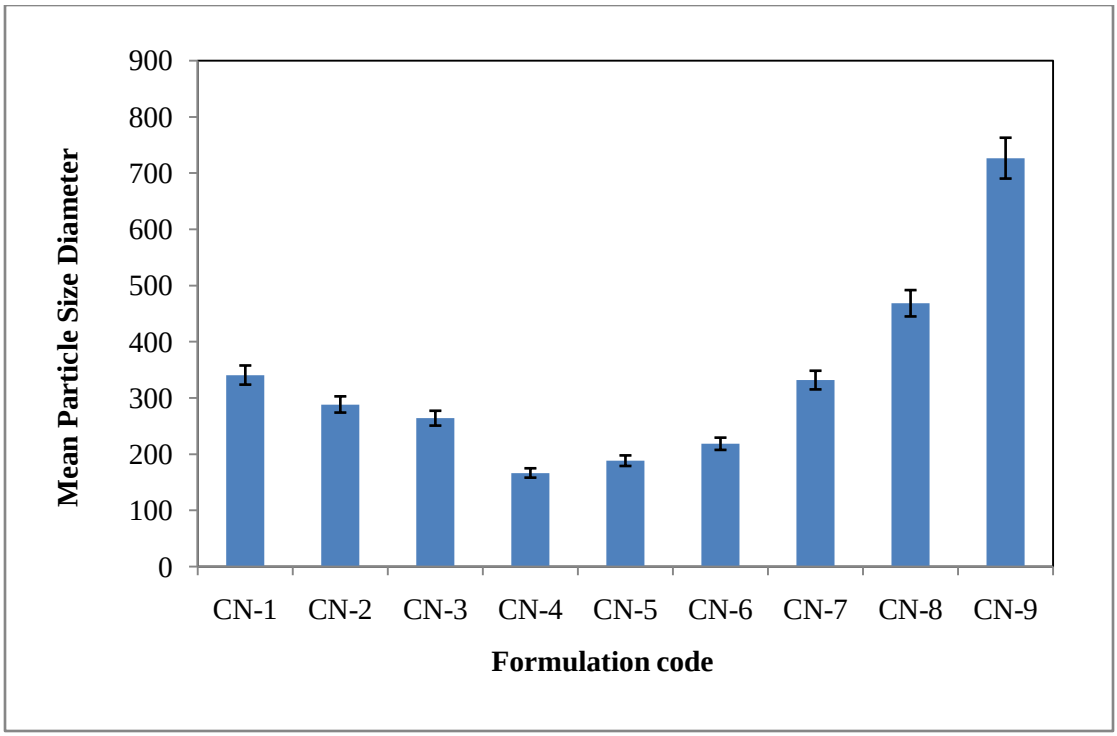

Fig 5.32. Particle size distribution of Capecitabine loaded nanoparticle formulations.

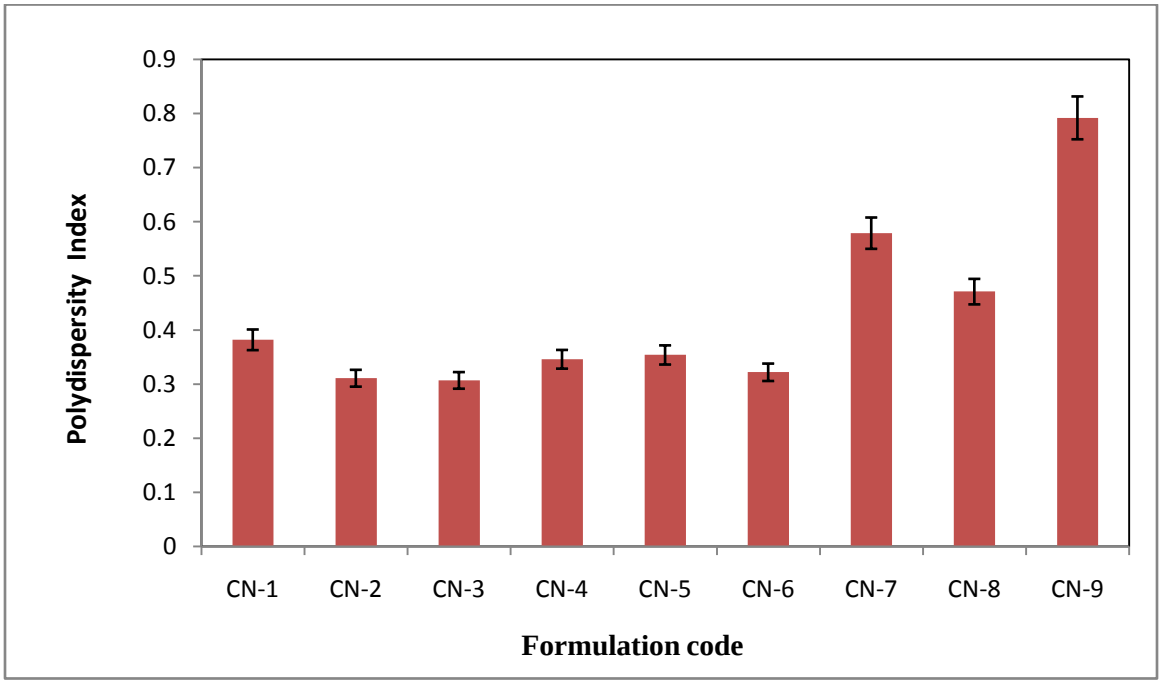

Fig 5.33. Polydispersity Index of Capecitabine loaded nanoparticle formulations.

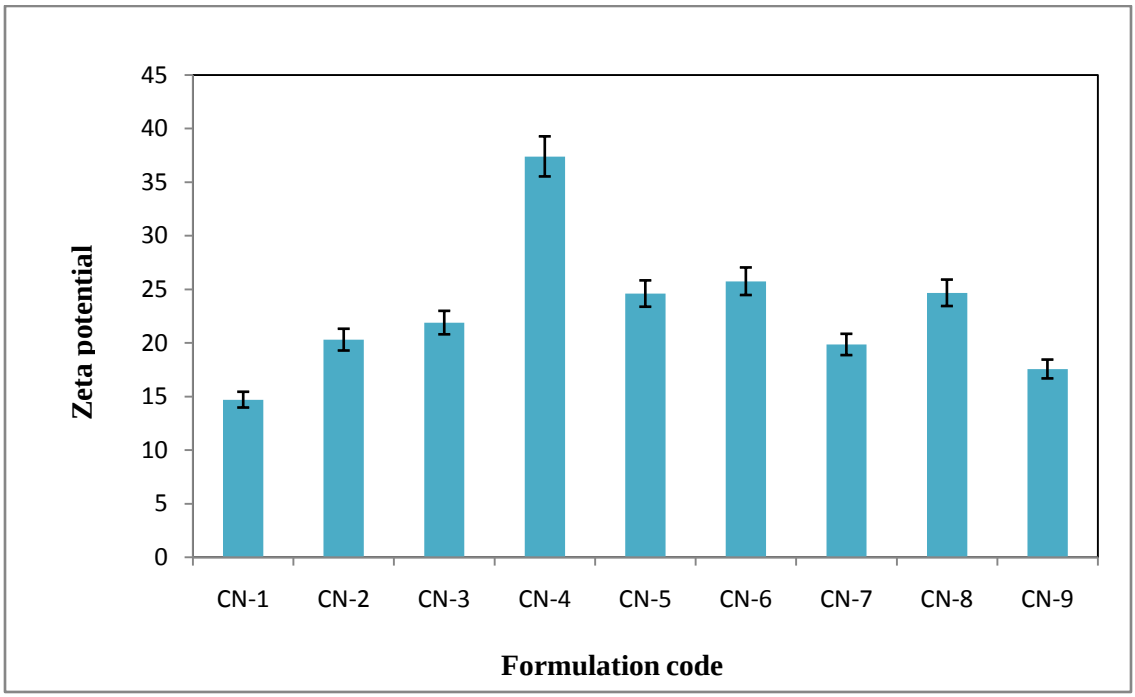

Fig 5.34. Zeta Potential (mV) of Capecitabine loaded nanoparticle formulations.

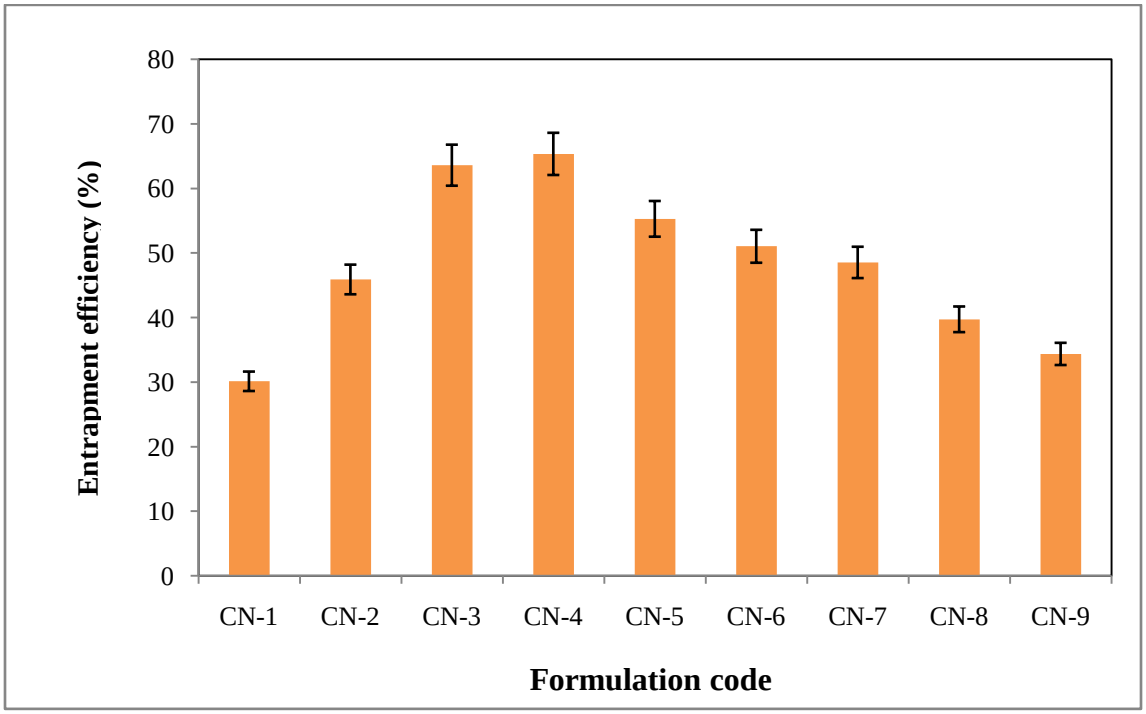

Fig 5.35.Entrapment efficiency (%) of Capecitabine loaded nanoparticle formulations.

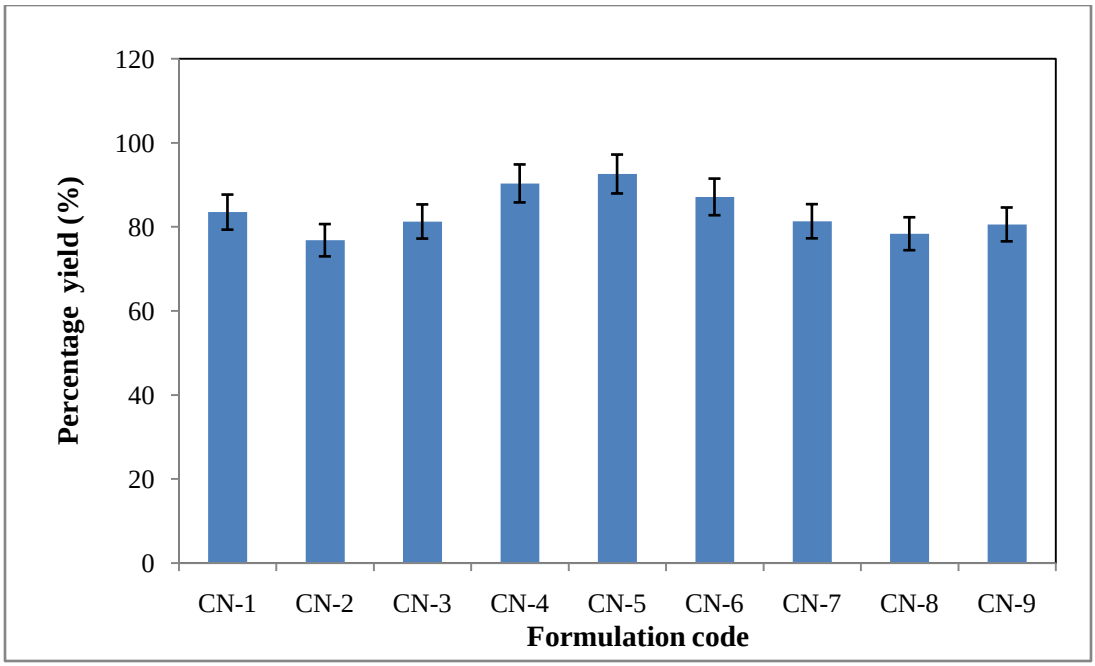

Fig 5.36.Percentage yield (%) of Capecitabine loaded nanoparticle formulations.

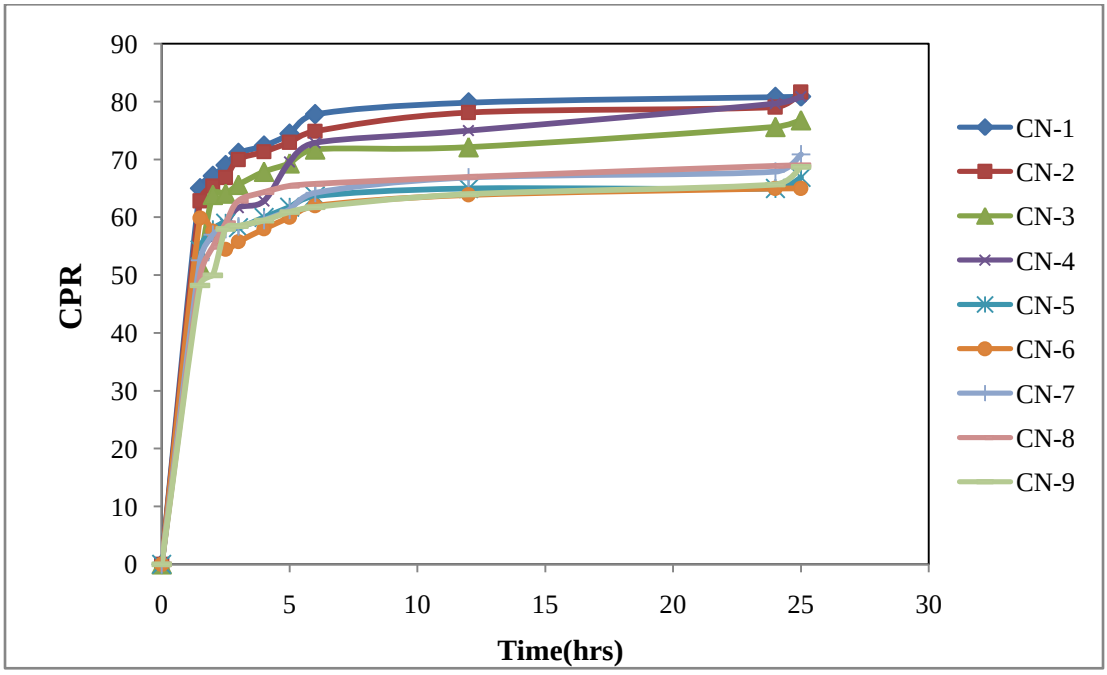

Fig 5.37.*In-vitro* drug release of Capecitabine nanoparticles in0.1MHCl (pH 1.2).

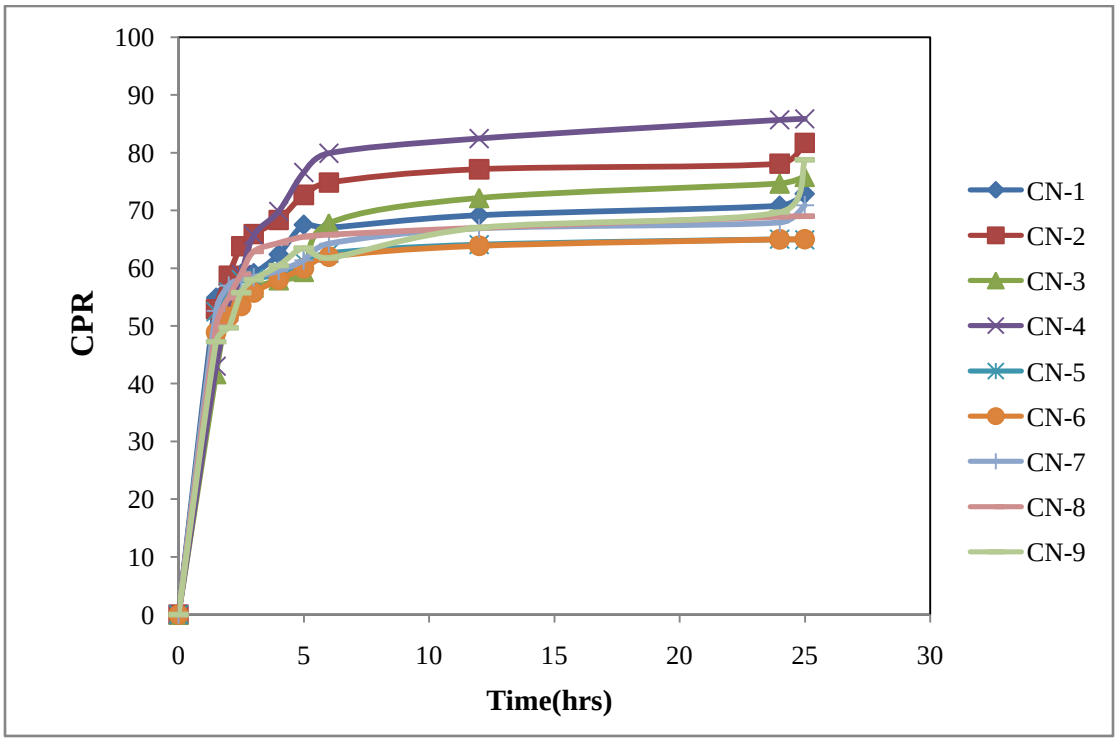

Fig 5.38. *In-vitro* drug release of Capecitabine nanoparticles in pH 6.8.

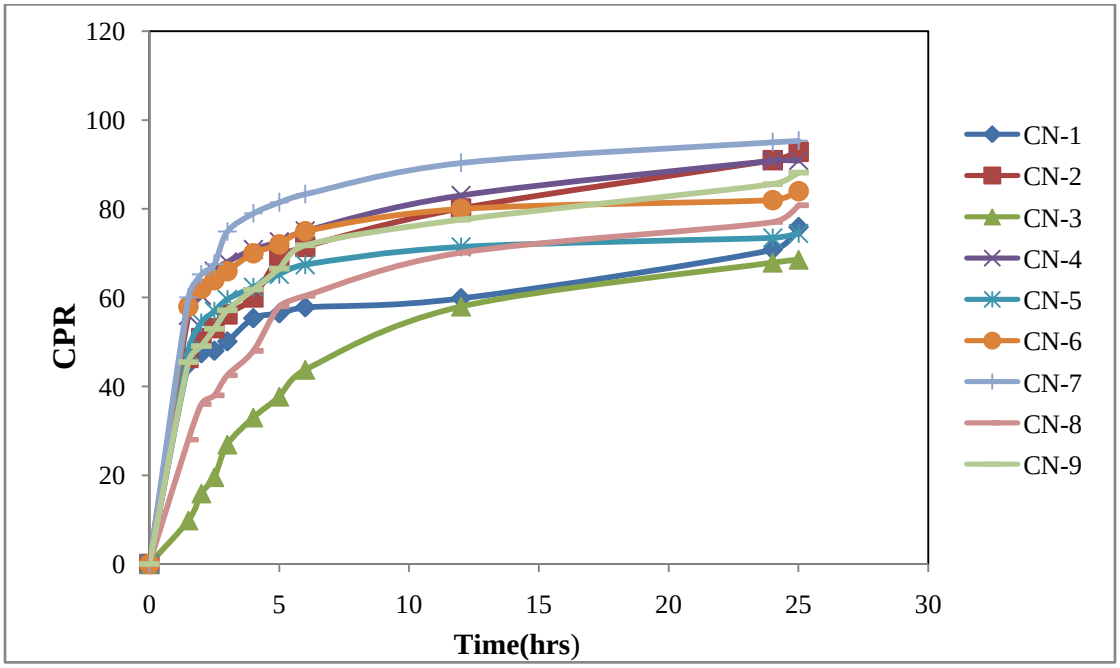

Fig 5.39.*In-vitro* drug release of Capecitabine nanoparticles in pH 7.4.

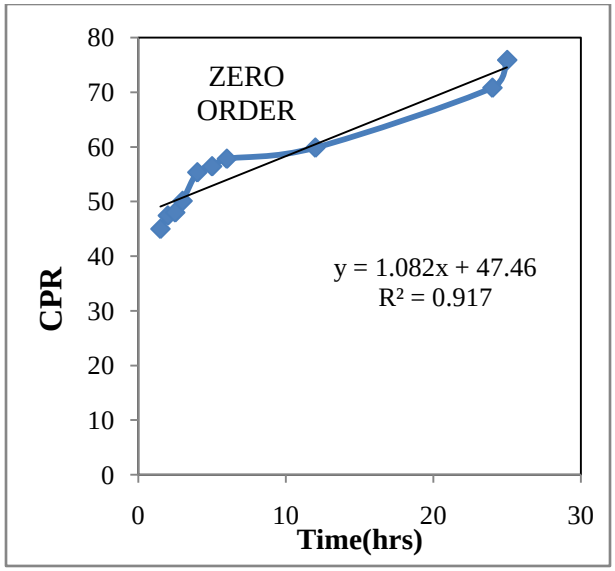

Fig 5.40.Zero order kinetics of CN-1.

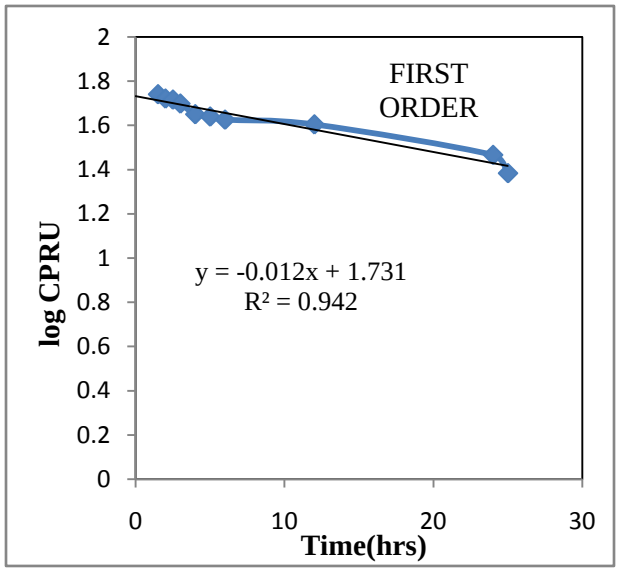

Fig 5.41. First order kinetics of CN-1.

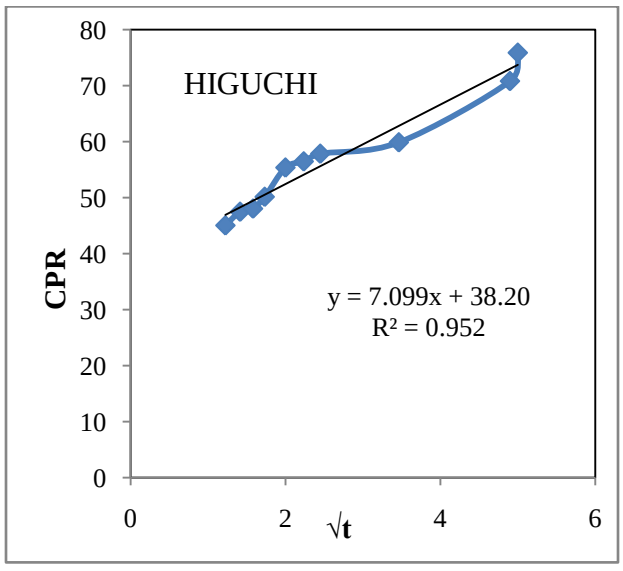

Fig 5.42. Higuchi kinetics of CN-1.

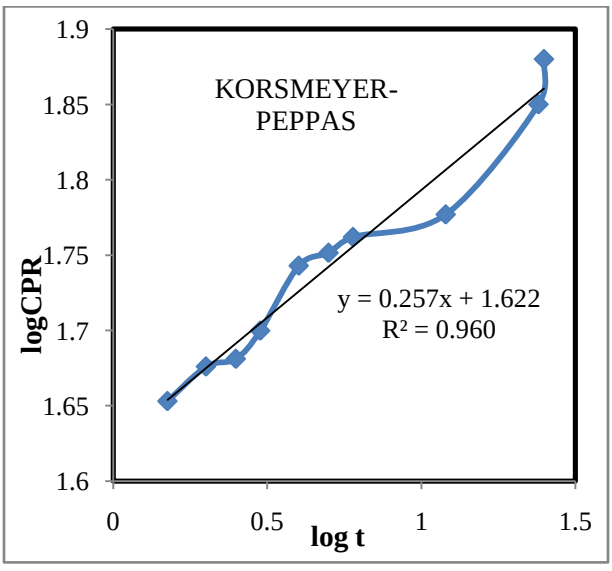

Fig 5 .43.Korsmeyer-Peppas kinetics of CN-1.

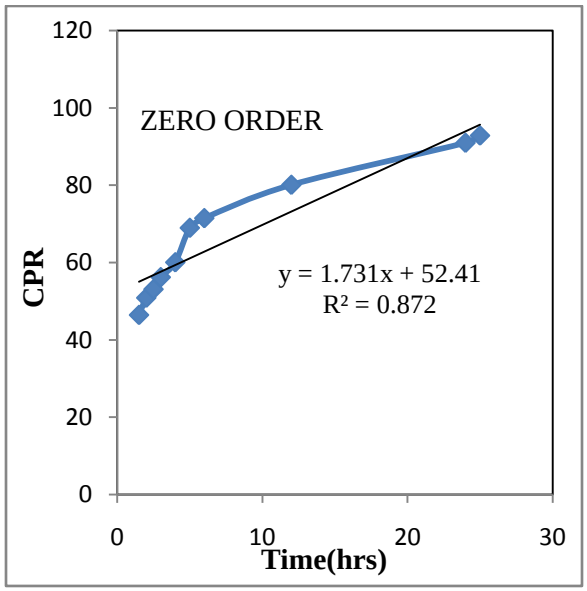

Fig 5.44.Zero order kinetics of CN-2.

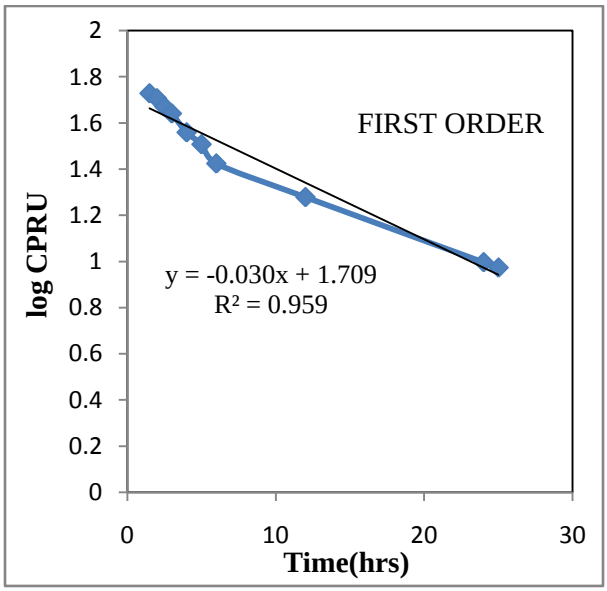

Fig 5.45. First order kinetics of CN-2.

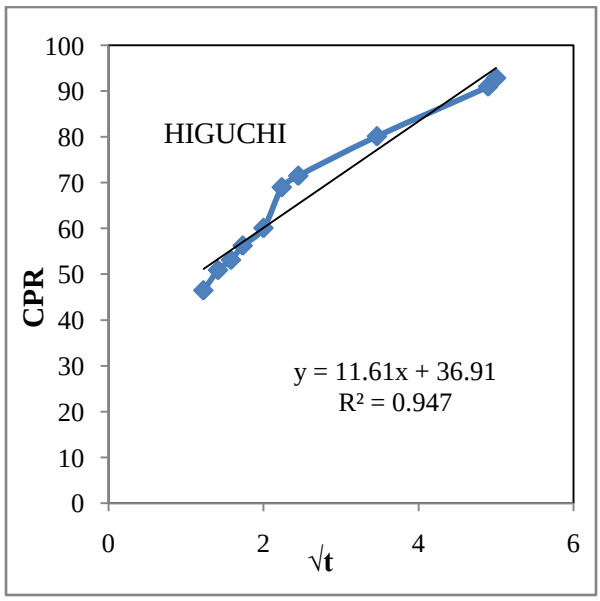

Fig 5.46.Higuchi kinetics of CN-2.

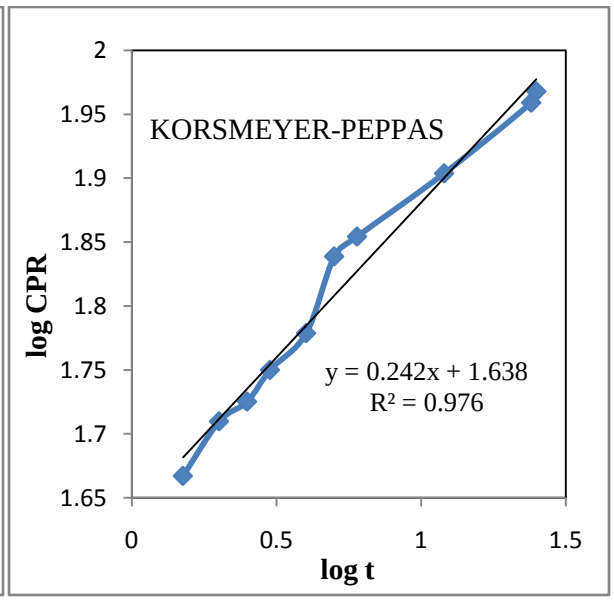

Fig 5.47.Korsmeyer-Peppas kinetics of CN-2.

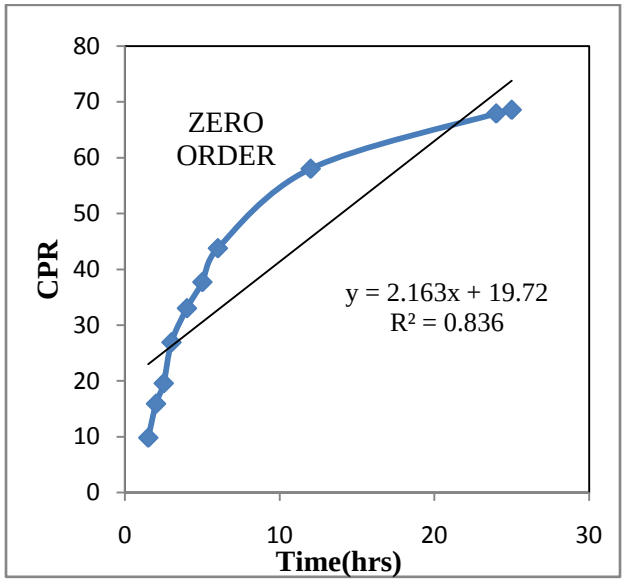

Fig 5.48. Zero-order kinetics of CN-3.

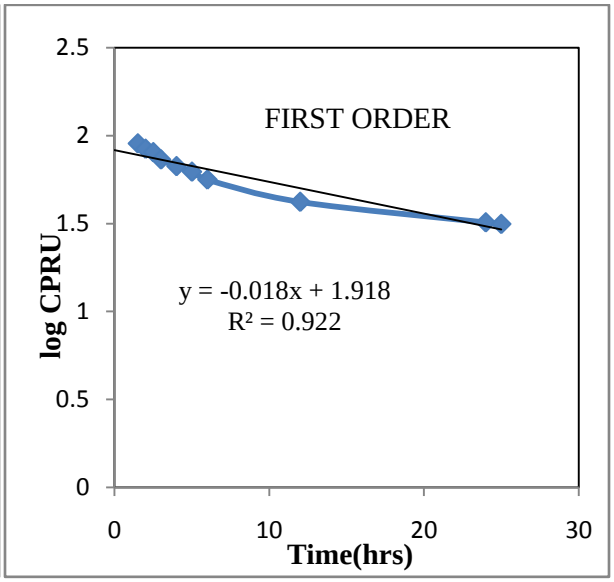

Fig 5.49. First-order kinetics of CN-3.

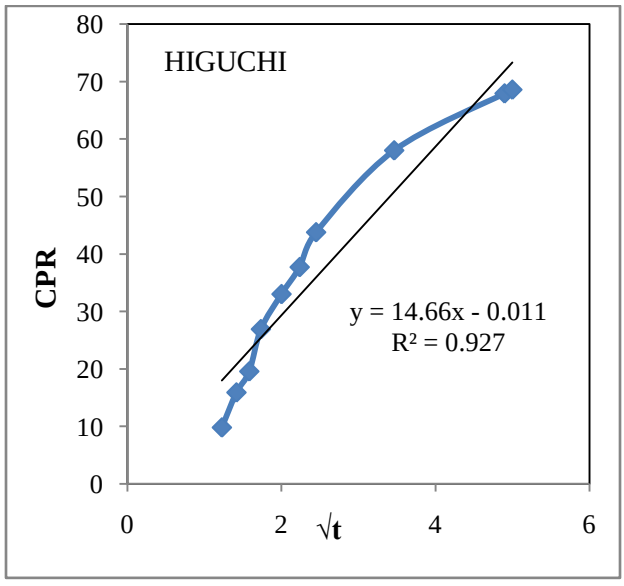

Fig 5.50. Higuchi kinetics of CN-3.

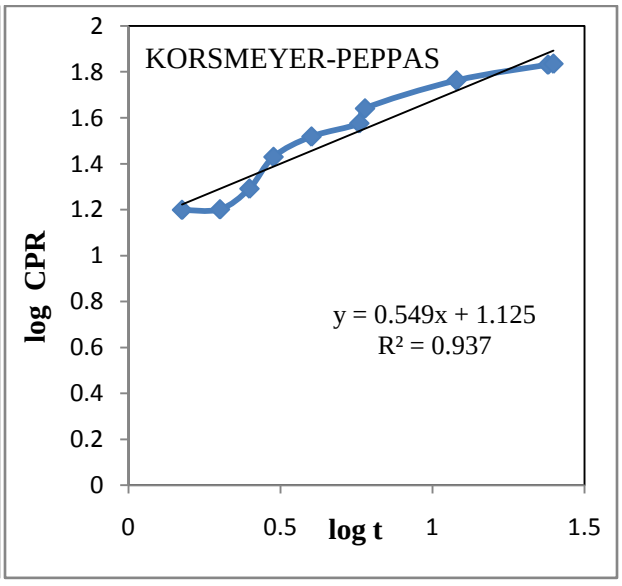

Fig 5.51. Korsmeyer-Peppas kinetics of CN-3.

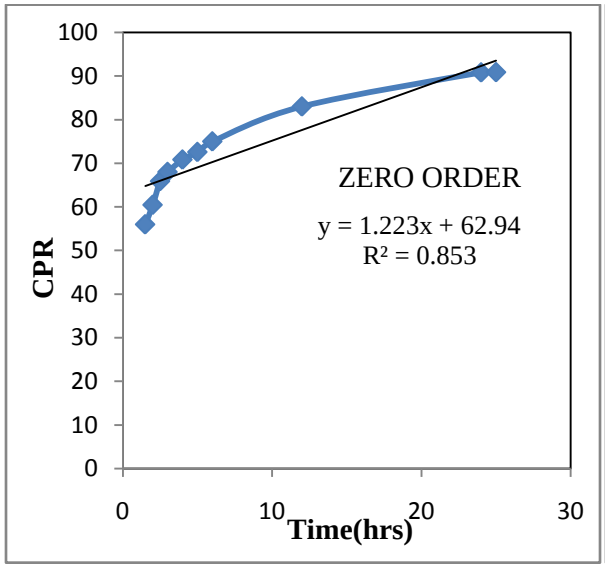

Fig 5.52. Zero order kinetics of CN-4.

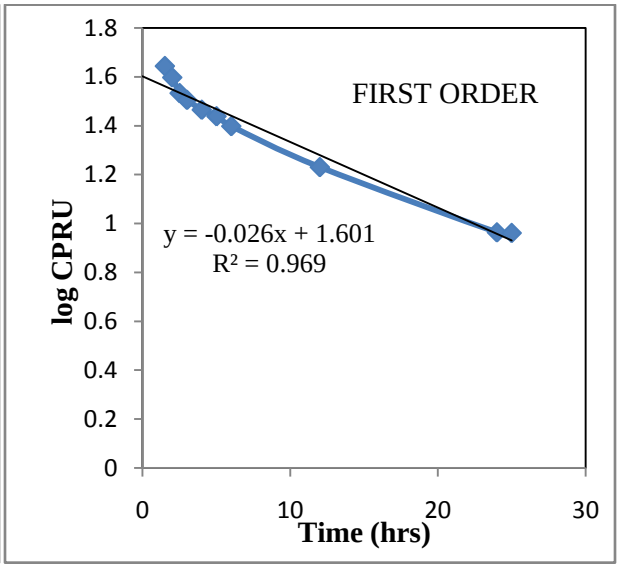

Fig 5.53. First order kinetics of CN-4.

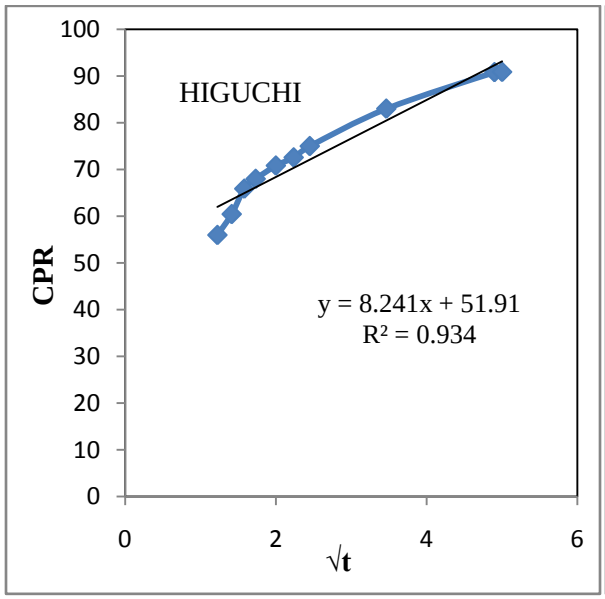

Fig 5.54. Higuchi kinetics of CN-4.

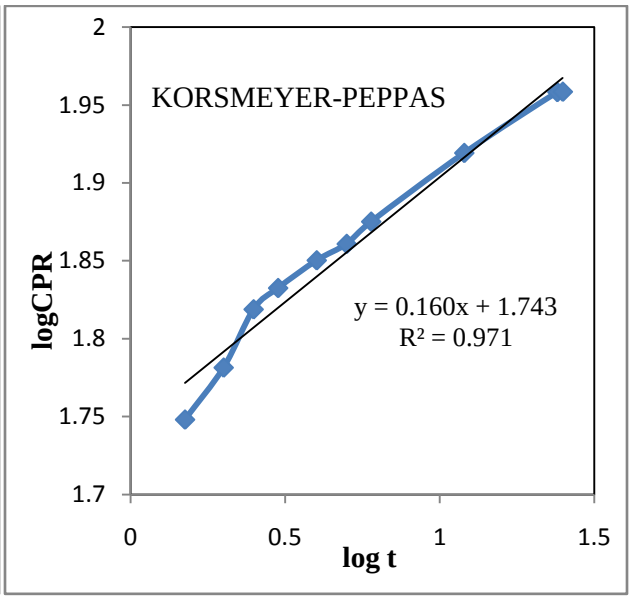

Fig 5.55. Korsmeyer-Peppas kinetics of CN-4.

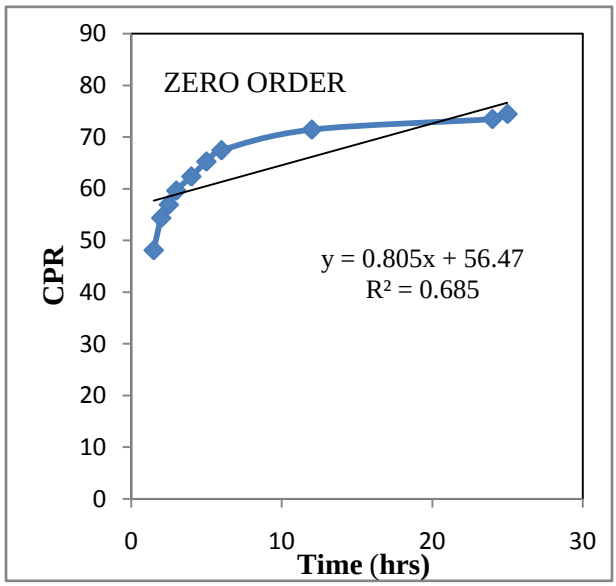

Fig 5.56.Zero order kinetics of CN-5.

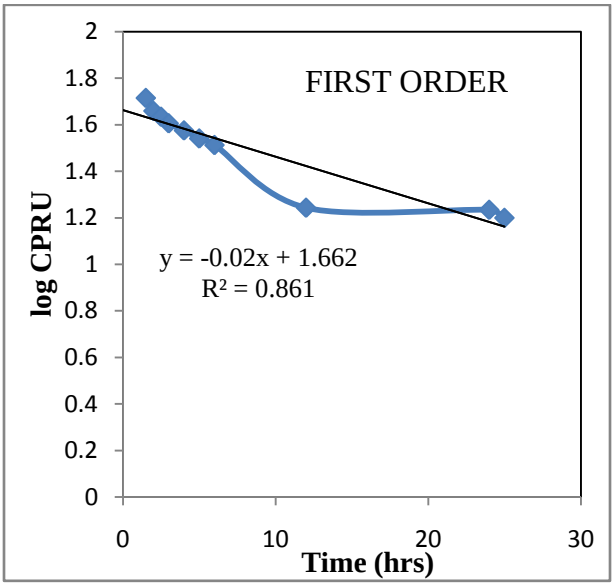

Fig 5.57. First order kinetics of CN-5.

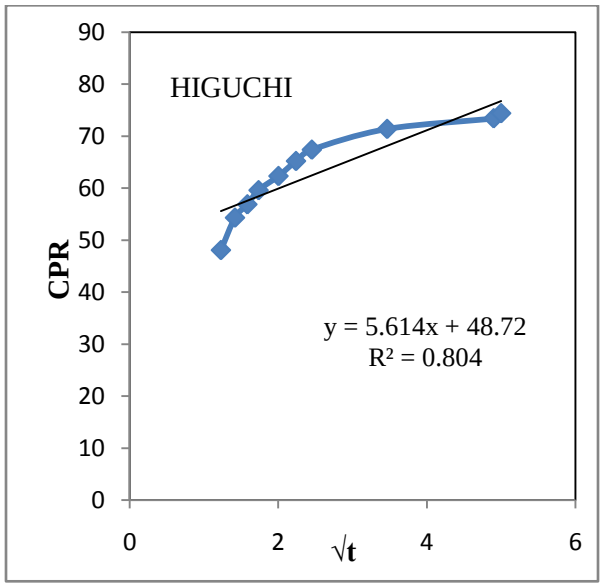

Fig 5.58.Higuchi kinetics of CN-5.

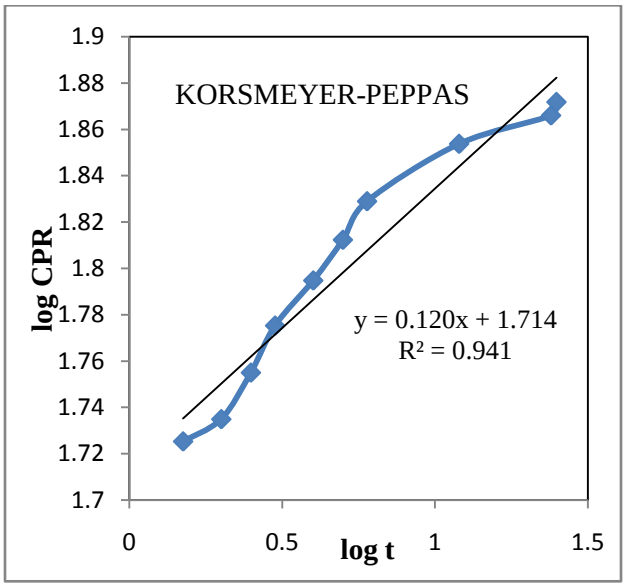

Fig 5.59.Korsmeyer-Peppas kinetics of CN-5.

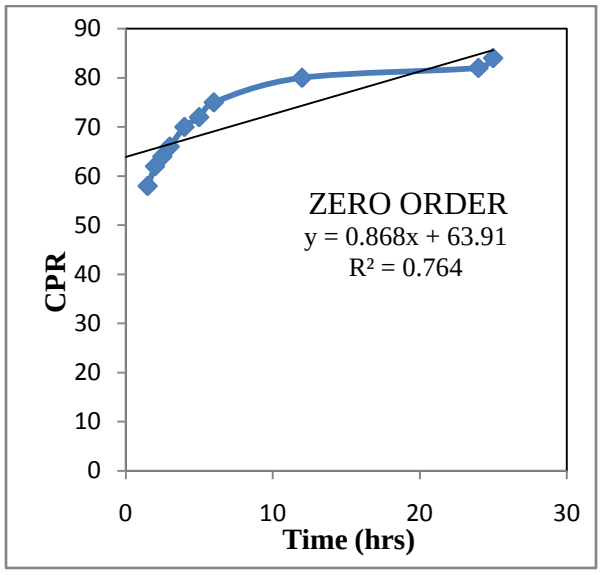

Fig 5.60.Zero order kinetics of CN-6.

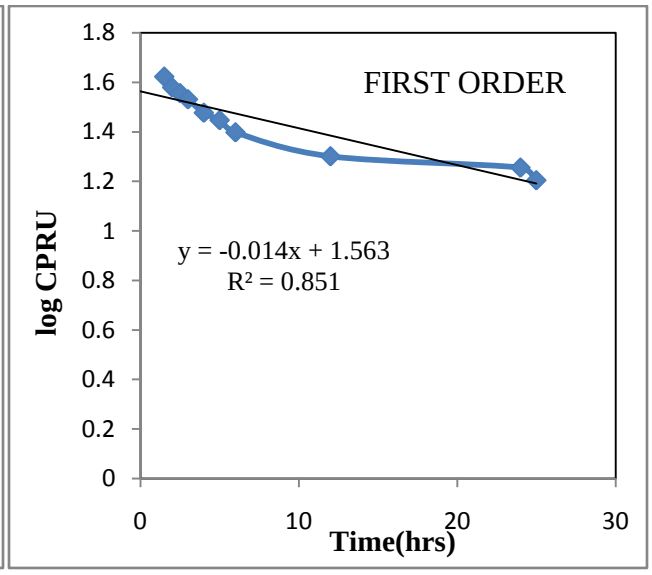

Fig 5.61. First order kinetics of CN-6.

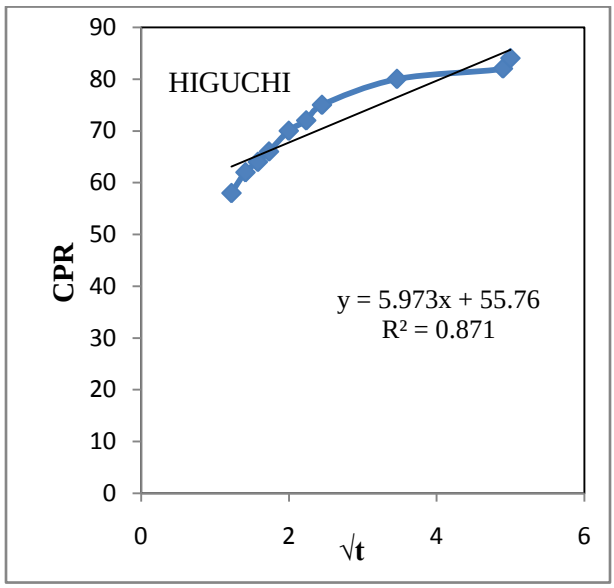

Fig 5.62.Higuchi kinetics of CN-6.

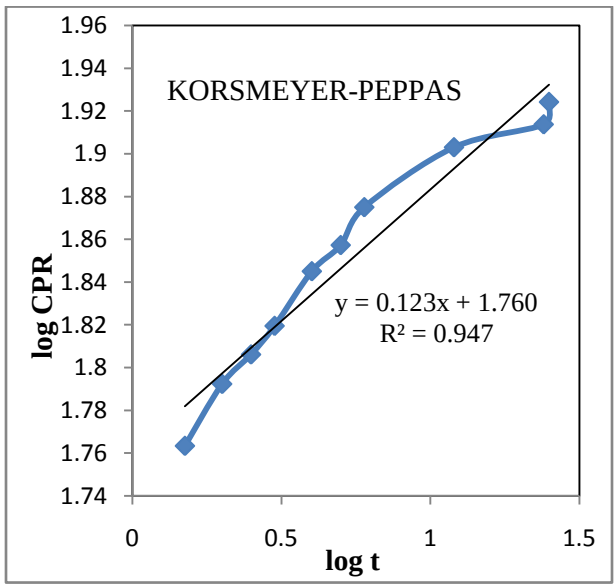

Fig 5.63.Korsmeyer-Peppas kinetics of CN-6.

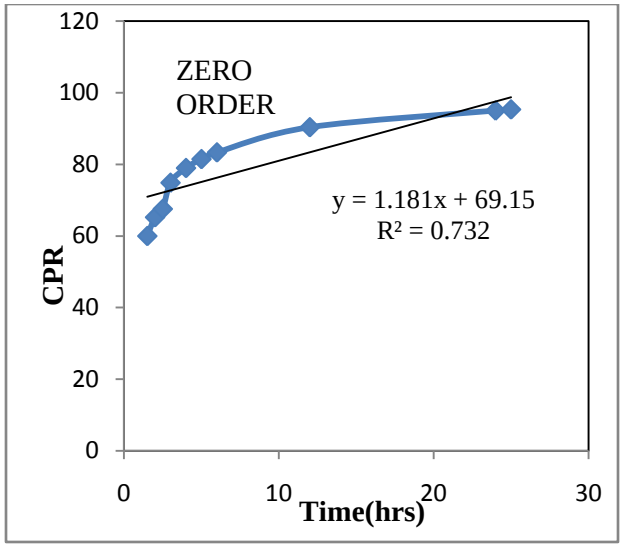

Fig 5.64.Zero order kinetics of CN-7.

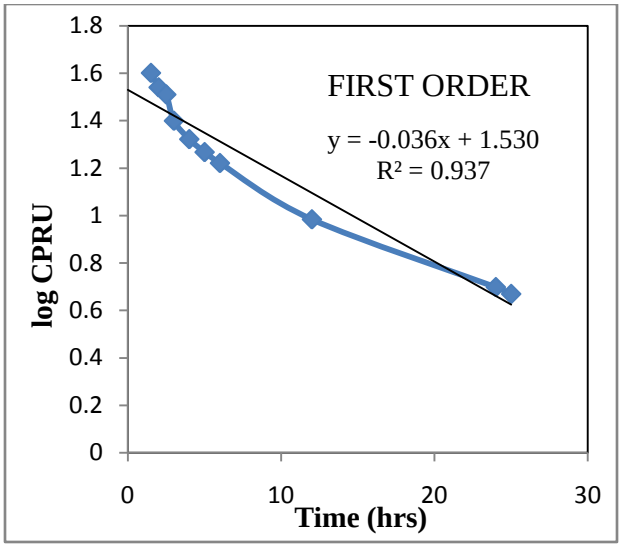

Fig 5.65. First order kinetics of CN-7.

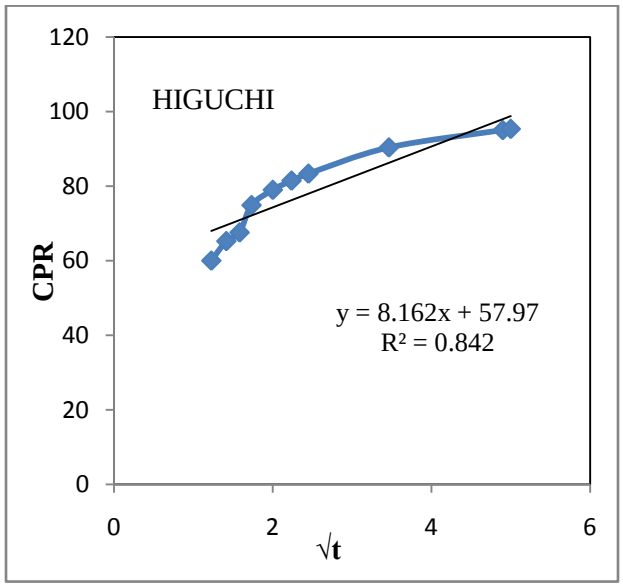

Fig 5.66.Higuchi kinetics of CN-7.

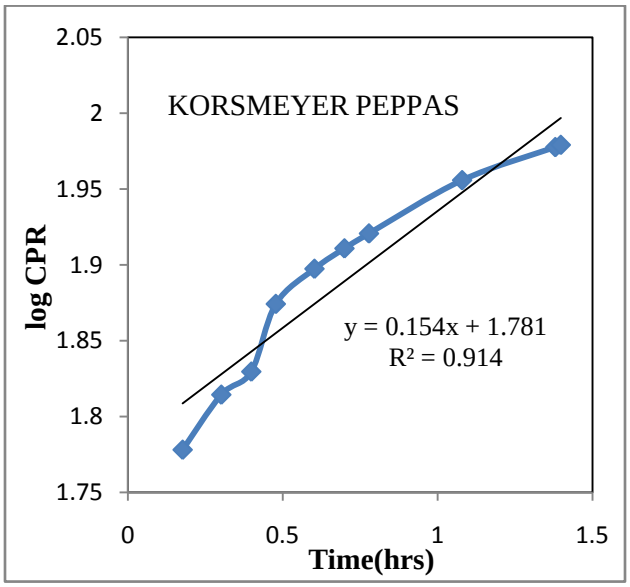

Fig 5.67.Korsmeyer-Peppas kinetics of CN-7.

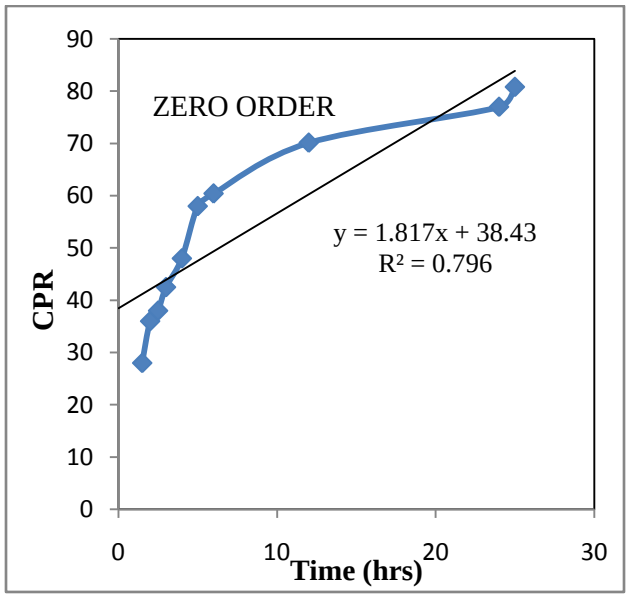

Fig 5.68.Zero order kinetics of CN-8.

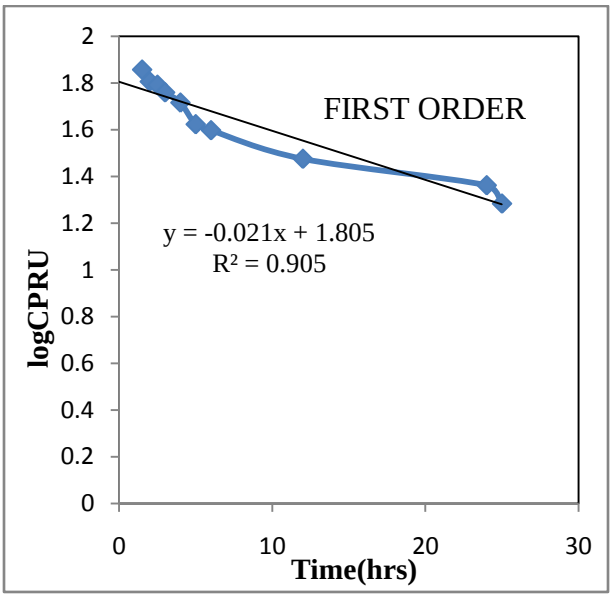

Fig 5.69. First order kinetics of CN-8.

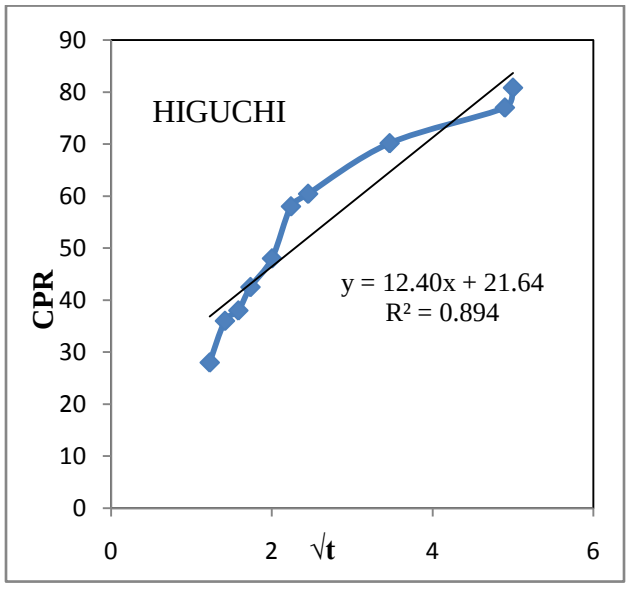

Fig 6.70. Higuchi kinetics of CN-8.

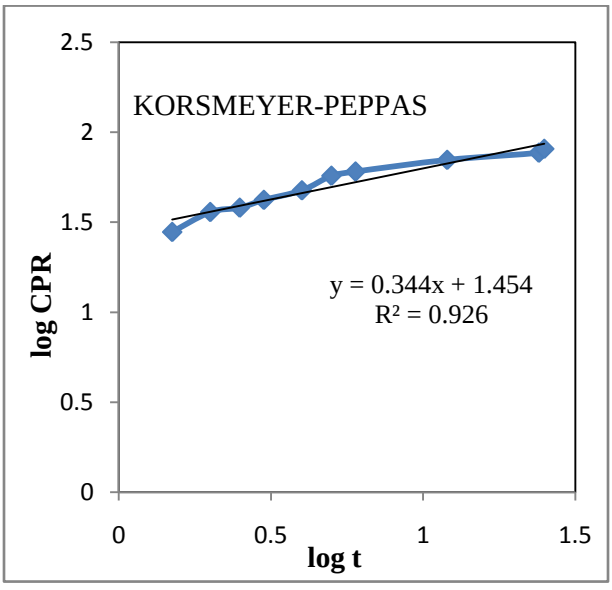

Fig 6.71.Korsmeyer-Peppas kinetics of CN-8.

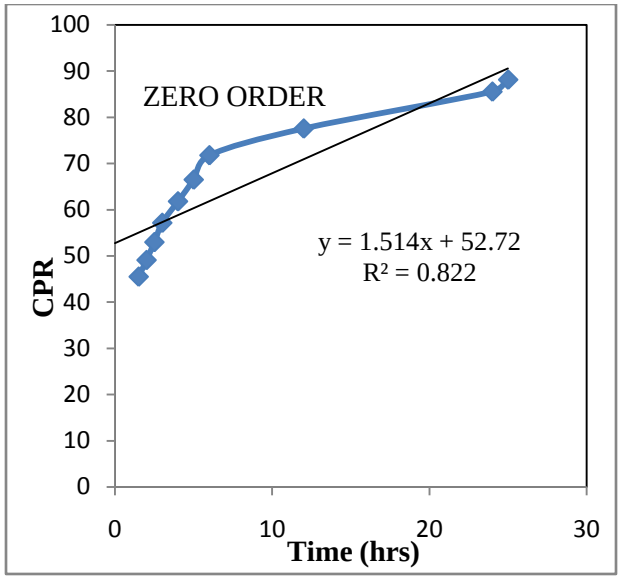

Fig 5.72.Zero order kinetics of CN-9.

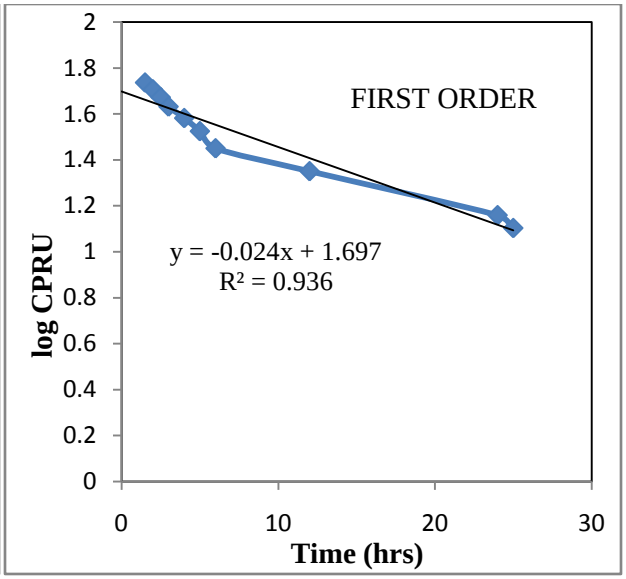

Fig 5.73. First order kinetics of CN-9.

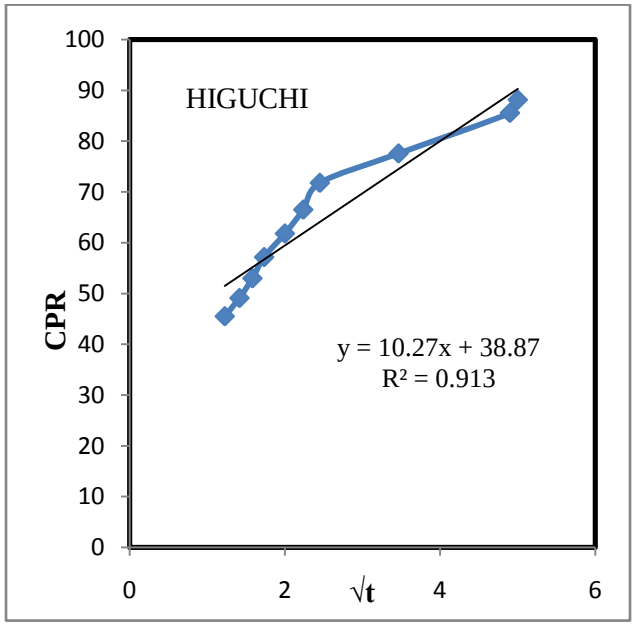

Fig 5.74. Higuchi kinetics of CN-9.

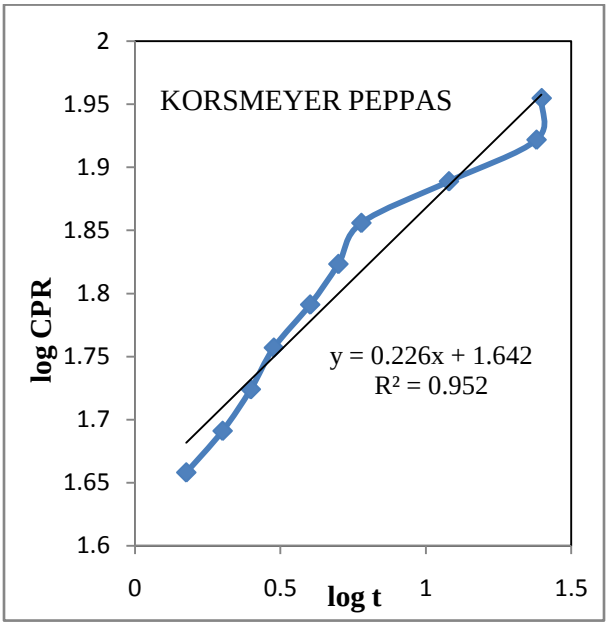

Fig 5.75. Korsmeyer-Peppas kinetics of CN-9.

CHAPTER 6:
RESULTS AND DISCUSSION

6. Results and Discussion

6.1. Drug-Excipient interaction study:

The drug-excipient interaction study revealed that Capecitabine did not have any physical interaction with chitosan and TPP, after a period of 6months in the humidity chamber, however physical interactions were observed in case of other excipients. The various drug-excipient interactions were tabulated in Table 5.1.

6.2. Drug-Excipient compatibility study:

Figure 5.1A and 5.1B represents the Fourier transform infra-red (FT-IR) spectrum of pure Capecitabine, excipient blend (LMW chitosan and STPP) and physical mixture of Capecitabine with the excipients, done at the beginning of the experiment and at the end of 6 months respectively. The FTIR spectra were compared amongst each other to detect the presence of any drug-excipient compatibility and stability problems. Capecitabine and the excipient blend preserved its characteristic peaks as was observed initially and after 6months, however for the physical mixture of Capecitabine with the excipients, the peaks had slightly flattened and widened after a period of 6months of storage in the humidity chamber, as compared to the initial peaks for the same. This widening of the peaks may be due to hydrogen bond formation between chitosan and Capecitabine. Since no significant changes were observed after comparing the FTIR spectra it could be assured that besides Capecitabine being compatible with the excipient blend both at the beginning of the experiment and after 6months, they were also stable individually. DSC analysis of Capecitabine with the excipient blend of chitosan and tripolyphosphate was also carried out to analyze the compatibility between Capecitabine and its excipients. It was found out from Fig 5.19 that Capecitabine, chitosan and TPP had retained its characteristic melting point at 123.72°C, 314.18°C and 602.56°C respectively, indicating that Capecitabine was compatible with both chitosan and sodium tripolyphosphate.

6.3. Characterization of Capecitabine:

6.3.1. Determination of λ_{max} of Capecitabine and preparation of calibration curve:

The diluted stock solutions of Capecitabine, prepared in Milli-Q water,0.1M HCl (pH 1.2),phosphate buffer pH 6.8 and phosphate buffer pH 7.4, were analyzed between 200-400 nm and were measured after standing for 5 minutes at λ_{max} of each medium. λ_{max} of Capecitabine in Milli-Q water, pH 1.2, pH 6.8 and pH 7.4 were found to be 215nm, 214nm, 215nm and 215nm respectively(Fig 5.2,Fig 5.3,Fig 5.4 and Fig 5.5).The similar values of λ_{max} indicates that there was no degradation of the drug in different medias. R^2 values of the calibration curves of Capecitabine in different buffer solutions were 0.997, 0.995, 0.999 and 0.999 (Fig 5.6, Fig 5.7, Fig 5.8, and Fig 5.9).

6.3.2.Fourier transform infra-red (FT-IR) spectroscopy:

The received gift sample of Capecitabine was identified by Fourier transform infra-red spectroscopy and was compared with that of reference in IP 2014 (Fig 5.10).The spectra was found to be similar as shown in Fig 5.11.,confirming the identity of the sample.

6.3.3.Differential Scanning Calorimetry (DSC):

The received gift sample of Capecitabine was identified by Differential Scanning Calorimetry. Capecitabine showed a strong endothermic peak at 120.44°C, confirming the presence of a single crystalline form of Capecitabine, as shown in Fig 5.12.

6.4. Characterization of Chitosan:

6.4.1. Fourier transform infra-red (FT-IR) spectroscopy:

Chitosan was analyzed by Fourier transform infra-red (FT-IR) spectroscopy. Fig 5.13 shows the FT-IR spectrum of Chitosan. N-H Stretching, O-H Stretching, C=O Stretching of Amide were observed at 3624.04092 cm^{-1},3234.4416 cm^{-1} and 1654.73146 cm^{-1}respectively and NH bending and C-O-C Stretching were observed at 1577.1526 cm^{-1} and 1151.74766 cm^{-1} respectively.

6.4.2. Differential Scanning Calorimetry (DSC):

Figure 5.14 represents the DSC curve of LMW chitosan. An exothermic peak was observed at 309 °C which is characteristic for LMW chitosan and indicates the presence of one crystalline form of Chitosan.

6.5. Characterization of chitosan-tripolyphosphate nanoparticles:

6.5.1. Particle Size, polydispersity index (PDI) and zeta potential:

The particle size, PDI and zeta potential of each nanoparticle formulation were tabulated in Table 5.2.The average particle size of the nanoparticles ranged from 166.5 nm (CN-4) to 726.4 nm (CN-9) with a PDI of 0.346 and 0.792respectively.CN-4 was selected as the optimized formulation owing to its uniform particle size and excellent PDI value. The zeta potential of CN-4 was observed to be + 37.4 mV, indicating the cationic property of Chitosan in the nanoparticles and also the stability of the suspension. The increase in particle size and PDI of the nanoparticles with increase in concentration of chitosan was because of the increase in strong inter-molecular hydrogen bonding interactions between the chitosan molecules [63].that lead particles of the dispersion to agglomerate and cause disturbance in the homogenicity of the system. The effect of varying chitosan concentration on the particle size, PDI and zeta potential of nanoparticles is represented in Fig 5.32, 5.33 and 5.34 respectively.

6.5.1.1. Effect of polymer concentration:

The optimum amount of chitosan was found to be 2.9 mg (CN-4)as it showed the lowest effective diameter and excellent polydispersity index.The zeta potential of CN-4 was found to be + 37.4 mV, suggesting the nanoparticle suspension to be stable. With increase in polymer concentration, the heterogenecity of molecular weight increased which caused higher effective diameter and non uniformity. The particle size distribution and zeta potential of CN-4 and CN-5 nanoparticle formulations were shown in Fig 5.28 to Fig 5.31.

6.5.2. Entrapment Efficiency (%):

The Entrapment efficiency (%) of each nanoparticle formulation was tabulated in Table 5.3.The entrapment efficiency (%) varied with increasing chitosan concentration, while keeping the TPP concentration constant. It was also observed that CN-4 had the maximum entrapment efficiency of 65.34% on the basis of which it was taken for further characterization. The effect of varying chitosan concentration on the Entrapment Efficiency (%) of nanoparticles was observed in Fig 5.35.

6.5.3. Percentage Yield (% yield):

The Percentage yield (%) of each nanoparticle formulation was tabulated in Table 5.3.The product yield (%) varied with increasing chitosan concentration, while keeping the TPP concentration constant.CN-4 had a percentage yield of 90.32%. The effect of varying chitosan concentration on the Entrapment Efficiency (%) of nanoparticles was observed in Fig 5.36.

6.5.4.Fourier transform infra-red (FT-IR) spectroscopy:

It was observed that the spectrum of blank chitosan tripolyphosphate nanoparticles (Fig 5.15) and Capecitabine loaded chitosan-tripolyphosphate nanoparticles (Fig 5.16) were different from that of pure Chitosan matrix (Fig 5.13).The wave number range from 1500 cm^{-1} to 3600 cm^{-1} was different among the two spectra. The peak of 3523.5 cm^{-1}, became wider and flatter; indicating that hydrogen bonding was enhanced (Rodgers *et al*., 2006). The peaks of amide I and amide II in chitosan-TPP nanoparticles shifted to 1645.76cm^{-1} and 1557.67cm^{-1} respectively, due to electrostatic interaction between phosphoric groups of TPP and amino groups of chitosan in nanoparticles.

6.5.5. Differential Scanning Calorimetry (DSC):

Figure 5.17 and 5.18 represents the DSC curve of blank chitosan-tripolyphosphate nanoparticles and Capecitabine loaded nanoparticles (CN-4) respectively. Two endothermic peaks were observed at 70.82 °C and 324.22°C, for the blank chitosan-tripolyphosphate nanoparticles. Two endothermic peaks were observed at 59.22°C and 144.46°C.No peaks were observed near 300°C in Capecitabine loaded nanoparticles (CN-4). No polymorphism was observed due to the interaction between Capecitabine and the excipients used in the preparation. Since an endothermic peak was observed at 144.46°C, it could be concluded that Capecitabine was present in the nanoparticles in its crystalline form. The differences observed between the thermograms of pure polymers and the nanoparticles may indicate the presence of strong ionic interactions which could contribute to the formation of new structural entity with specific thermal characteristics.

6.5.6. Scanning Electron Microscopy (SEM):

Fig 5.20 to Fig 5.23 represents the SEM images of freeze dried Capecitabine loaded nanoparticles (CN-4 and CN-5) captured at different magnifications. The increase in particle size after freeze drying could be attributed to agglomeration due to strong inter- and intra-molecular hydrogen bonding interactions between the nanoparticles, which became dominant over particle-particle repulsion, during freeze drying operation [63].

6.5.7. Transmission Electron Microscopy (TEM):

For the exploration of surface morphology, the transmission electron microscope study was performed on the prepared formulations of Capecitabine loaded nanoparticles (CN-4 and CN-5).This study revealed that particles were spherical in shape, smooth and had a non-porous surface as shown in Fig 5.24 to Fig 5.27.The discrepancy in the size of chitosan nanoparticles between DLS and TEM can be that chitosan nanoparticles swell in aqueous media and DLS gives a hydrodynamic diameter of nanoparticles, while TEM gives an actual diameter of nanoparticles in dry state. It was also noticed that these nanoparticles had a deeper colour in core and surface indicating that these regions have higher electron density distribution. As TPP contains phosphorous element, which has a higher electron density than those elements of chitosan, so it can be inferred that chitosan has higher degree of cross-linking density with TPP in these regions. Capecitabine is assumed to be present at these deeper regions of cross-linking sites.

6.5.8. *In Vitro* drug release study:

It was observed that in case of all the formulations, almost 80%-90% of Capecitabine was released from the nanoparticles within 24 hours. The cumulative percentage release of Capecitabine in different pH environments, at the end of 24 hours is given in Table 5.4 to Table 5.6. Almost 45-65% of Capecitabine was released from the nanoparticles, during the initial burst release which lasted for a period of 1.5 hours. It was followed by a slower steady release rate of the drug which gradually became linear with time (Figure 5.37 to Fig 5.39).The cause of initial burst release was due to the rapid dissolution of the drug molecules located at the surfaces of the nanospheres [64].The differences observed in the in-vitro release profile of Capecitabine in

various pH was due to the property of Chitosan to swell in acidic media and its insolubility in alkaline media. It was also observed that the rate of dissolution declined upon increasing the concentration of chitosan in the formulation. This was because, with increase in concentration of the polymer, the nanosphere thickness increases as more than one chitosan molecule gets involved in the cross linking of a single particle. As a result the drug that gets embedded within the cross linked matrix of the polymer has to overcome a denser network of cross links to get dissolved in the dissolution fluid.

6.5.9.Evaluation of drug release mechanism by various kinetic models:

Various kinetic models were employed to investigate drug release mechanism of the formulations using *in vitro* dissolution data. The *in vitro* release data were fitted to models representing Zero-order, First-order, Higuchi's square root of time and Korsmeyer-Peppas model to determine the correlation coefficient, slope and intercept values (Fig 5.40 to Fig 5.75). Various parameters of the model equations were tabulated in Table 5.7. From the values of the correlation coefficients, the best fitted data can be predicted. The curve fitting of the release data was carried out mainly by regression analysis. In spherical matrices, if $n \leq 0.43$, a Fickian (case I), if $0.43 < n < 0.85$, a non-Fickian and if $n \geq 0.85$, a case-II (zero order) drug release mechanism dominates. The maximum correlation coefficient has been considered as statistical parameter to designate the function with the best fit to the data.

The examination of correlation coefficient (R^2) values indicated that the drug release followed Korsmeyer-Peppas model, because the correlation coefficient value obtained for this model was the highest as compared to the R^2 values of zero-order, first-order and Higuchi's model. This means that the release of Capecitabine from the polymeric nanoparticles followed diffusion kinetics. The data are supportive to the findings that a water soluble drug incorporated in the swellable matrix device is mainly released by diffusional mechanism. To characterize the type of diffusion, the value of the release exponent 'n' was analyzed from the graph of log cumulative % drug release vs.log time(Korsmeyer-Peppas model), for different Capecitabine loaded nanoparticle formulations. The values for 'n' were in the range of 0.120 to 0.549 as obtained from the slope of the graphs.

CHAPTER 7:
CONCLUSION

7. Conclusion

Chitosan nanoparticles containing Capecitabine were successfully prepared by ionotropic gelation method, using Sodium tripolyphosphate as the cross-linking agent. The method of preparation was found to be simple, non-toxic, organic solvent free, convenient and cheap.

Capecitabine was found to have no physical interaction with chitosan and tripolyphosphate, and that Capecitabine was also compatible with chitosan and TPP was confirmed by FT-IR and DSC studies, performed to test the compatibility between drug and polymer. The mean particle size of the formulations was between 166.5 nm to 726.4nm, with polydispersity index within the range of 0.307 to 0.792, depending upon the concentration of the polymer. The zeta potential of the nanoparticles also varied accordingly with the change of polymer concentration.

The Capecitabine loaded chitosan-tripolyphosphate nanoparticles had a maximum entrapment efficiency of 65.54% with a maximum percentage yield of 92.56%.

The optimized formulation was particularly CN-4, which was characterized further by FT-IR, DSC, SEM and TEM. CN-5 was also chosen for SEM and TEM studies owing to its uniform particle size distribution and excellent polydispersity index.

In vitro release study was done in simulated gastric fluid (phosphate buffer pH 1.2), simulated intestinal fluid (phosphate buffer pH 6.8) and in phosphate buffer pH 7.4. It was found that almost 65%, 50% and 45% of Capecitabine was released after 5 hours in simulated gastric fluid (phosphate buffer pH 1.2), simulated intestinal fluid (phosphate buffer pH 6.8) and in phosphate buffer pH 7.4, respectively. Thereafter, the release became sustained upto a period of 24 hours.

Kinetic modeling studies of the drug release behavior showed Fickian diffusion of the drug from the formulations.

The results of the present study clearly confers promising potentials of chitosan nanoparticles for delivering an anti-cancer drug like Capecitabine to tumor tissues and also as a potential alternative to conventional dosage form as the sustained release will be effective for reducing the

dosing frequency. As chitosan is biodegradable and biocompatible it is expected that the use of chitosan in formulation will not have any deleterious effect or toxic response in our body even if the nanoparticles are used for prolonged periods.

CHAPTER 8:
FUTURE SCOPE

8. Future Scope

The impact of nanotechnology extends from science and technology to the field of medical, legal and environmental applications. The major benefits of nanotechnology includes improved manufacturing methods, water purification systems, energy systems,nanomedicines,better food production methods and large scale infrastructure auto-fabrication. The matured nanotechnology will reduce labour, land or maintenance requirements placed on humans. Within a few decades, healthcare will be revolutionized by combining nanotechnology with biotechnology to produce ingestible systems, that will not only be harmlessly flushed out from the body of the patient, but will also notify the physician of the type and location of diseased cells and organs in case of any health problems. The new concept of technology is so broad and pervasive that it can influence every area of technology and science in ways that are surely unpredictable. We are just seeing the tip of the ice-berg in terms of the benefits nanotechnology can bring. Through nanotechnology:

1. Healthcare could be easily available which can be cheap, quick and efficient.
2. Many innovative devices can be developed by rapid and flexible manufacturing cycle.
3. Nanometer scale traps can be constructed that will be able to remove pollutants from the environment and deactivate chemical warfare agents.
4. Computers with the capabilities of current workstations will be the size of a grain of sand and will be able to operate for decades with the equivalence of a single wrist watch battery.
5. Potential applications in the healthcare industries include drug delivery systems, artificial tissues and organs, engineered enzymes and nano-robots which can be programmed and controlled to target individual cells [65].

India's nanotechnology initiatives:

The Govt. of India has taken strong initiatives to boost nanotechnology research and development.

Department of Science and Technology (DST) unit on nanosciences, Indian Association of Cultivation Science, Kolkata is carrying research on nanocomposites.Department of material science, unit of nanoscience at S.N Bose national center for Basic Sciences, is

working on nanocrystals and their optical properties. Center for nanotechnology, Vellore Institute of Technology, Vellore has reported synthesis of metal nanoparticles under reflux and microwave heating.

Indian Institutes and Universities are also involved in a number of collaboration with various research centers, institutes and universities of other countries. The trilateral initiatives between India, Brazil and South Africa promote collaborative programs between the department of Science and Technology of three participating countries [66].

CHAPTER 9:
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