TARGETED DRUG DELIVERY OF ANTI CANCER DRUG BY APPLYING GASTRO RETENTIVE SYSTEMS AND ITS PHARMACOLOGICAL EVALUATION

THESIS

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The Tamilnadu Dr. M.G.R. Medical University
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DOCTOR OF PHILOSOPHY
(Faculty of Pharmacy)

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DECLARATION

I hereby declare that the thesis entitled "**Targeted Drug Delivery Of Anti Cancer Drug By Applying Gastro Retentive Systems And Its Pharmacological Evaluation"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, as partial fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY** (Faculty of Pharmacy) was completely carried out by me during the period 2004-2009 under the guidance of **Prof. (Dr.) G. SRINIVASA RAO,** M. Pharm., Ph.D., Director, Research and Development, Vels College of Pharmacy, Chennai, Tamilnadu, India. This work is original and has not formed the basis for the award of any diploma, degree, associateship, fellowship or other similar title.

(**K.KAVITHA)**

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INTRODUCTION

Drugs, as pure chemical substance are seldom used alone, but rather as dosage forms (mixtures with one or more additives) or drug delivery systems[1]. The successful drug therapy is critical to the drug delivery systems since they provide safe and convenient delivery of accurate dose of a drug. Today new delivery methods of drugs are at the forefront of development and are aimed at optimizing therapy while maximizing patient comfort, mobility and convenience.

In order to make a drug that is stable, efficacious, elegant, easy to administer and safe, the active drug substance is combined with various other pharmacologically inert substances. This combination is known as drug formulation and as dosage form when it is marketed. So dosage forms are means by which drug molecules are delivered to the desired site of action within the body. There are literally numbers of different ways in which medicine can be formulated for use by the patient. In some instances, the physical form of the drug dictates to a large degree the chosen formulation. In vast majority, however, the actual presentation will be derived to satisfy both technical and marketing considerations. Collectively, all types of dosage forms constitute what are commonly referred to as 'Drug delivery systems'.

The word 'new' in relation to drug delivery systems is a search for something out of necessary. The aim is to minimize the disadvantages associated[2] with existing dosage form as much as possible(Table 1). Credit for the development of dosage forms goes to Claudius Galen, a Greek pharmacist-physician in 2nd century A.D. He formulated many drugs, mainly of natural origin, commonly referred to as Galenics to meet the needs of the people in providing drug product that were convenient to use. The art of galenics remained almost unchanged until the industrial revolution in the 19th century, coupled with expanded demand of variety of drug and the dosage forms. At that time, the main purpose of dosage form was to improve production rate using industrial techniques and focused on organoleptic attributes of dosage forms, such as taste and appearance.

While organoleptic qualities must not be compromised, yet the pharmaceutical researches in the last few decades realized that there are other important properties to be assessed to ensure quality dosage form design[3]. The actual performance of most of the drugs in therapy is now known to be greatly affected by the method of presentation of the drug as delivery system to the patient (Table 2). It follows, therefore, that tablets or capsules should exhibit a definite rate and extent of absorption of drug into systemic circulation, tested by pharmacokinetic method in animals and finally in human volunteers. It has become obvious that drug delivery system must not only contain, the accurate amount of drug, but upon administration to the patient also release the drug to the system. This concept, known as bioavailability, is defined as the rate and extend to which a drug is absorbed and becomes available at the site of action.

Table 1.1: Some Examples of Conventional Drug Delivery systems

Type	Route	Form	Purpose
Tablet	Oral	Solid	Systemic effect
Capsule	Oral	Solid	Systemic effects
Oral liquids	Oral	Liquid	Systemic effects
Powders	Oral	Solid	Systemic effects
Injections	Parenteral	Liquid	Systemic effects
Ointments	Topical	Semisolid	Local effect
Pastes	Topical	Semisolid	Local effect
Eye drops	Intra ocular	Liquid	Local effect
Aerosols	Intra respiratory	Gas	Local and systemic effects
Suppositories	Rectal / vaginal	Solid	Local and systemic effects

The salient aspects of the conventional drug delivery systems including tablets, capsule, liquid orals, and eye drops is the fluctuation in plasma concentration between dosages. This fluctuation in the plasma drug concentration can cause undesirable side effects with drug that have narrow therapeutic indices. There are

two ways to improve such a situation: first, the development of a new, better and safer drug and second, more effective and safer use of existing drug through development of a new drug delivery systems. The former is known as new chemical entity, NCE research and the latter improved chemical entity through new delivery search. The first approach, i.e., the development most new drug (NCE) takes 10 to 14 years at an enormous cost of over US$ 500 million and only one of every ten drugs researched and developed actually makes it to the market, which, therefore, resulted in the increased interest in the second approach.

The concept of new drug delivery systems represents a means by which drug may be continuously delivered either locally or systemically or a target site in an effective and repeatable manner. Controlled and targeted drug delivery systems have been receiving more and more attention as new methods of drug delivery.

Table 1.2: Changing Perception of Drug Delivery Systems

Periods	Terms	Emphasis in Drug Design
2nd century A.D.	Galenics	Convenient administrable drug form
Up to 19th century	Dosage Forms	Elegance, taste and stability when stored under reasonably adverse condition
Up to Mid-20th century	Drug delivery systems	A vehicle for delivering the drug to the patient that is effective, stable and safe
1960 – 1990	New drug delivery systems (incorporating concept of sustained and controlled release systems)	Optimizing therapeutic effectiveness while maximizing patient comfort and convenience
Post 1990	Novel drug delivery systems and Targeted Drug Delivery Systems	Delivering the drug directly to target tissue, thereby increasing potency, minimizing adverse reactions and reducing costs

Although “Sustained release” and “controlled release” terms are used interchangeably, albeit sustained release describes release of drug over extended period of time and controlled release means a system in which the release is

precisely controlled. Clearly, pharmaceutical experts would like all sustained release forms to be controlled. i.e., controlled delivery is the goal for all delivery systems. In sustained release product, therapeutic blood levels of drug is achieved by providing drug in a slow first order release. On the other hand, controlled release is accomplished by zero order release from product[4]. Examples of new drug delivery systems for various drugs have been developed, and a few are on the market or awaiting approval, is presented in Table 3.

TARGETED DRUG DELIVERY SYSTEM

One of the most exciting controlled release systems is the target-organ oriented drug delivery system. Presenting drugs into whole body is not only a waste but also likely to lead to harmful effects that can be eliminated if the drug is delivered only to specific target organ. Targeted delivery[5, 6] is not restricted to and one route of administration. Oral formulations, parenterals, transdermal and pulmonary route, and many other routes are available for effective drug targeting (Table 4). One of primary applications of targeted delivery is for treatment of tumor cells that involve highly cytotoxic drugs so that side effects and damage to healthy tissues are minimized. Targeting is done using ligands that range from simple conjugates to complex systems, directly coupled to the drug or to the moiety that encapsulates the drug such as liposome. Targeting can also be done by changing the formulation in a way that alters its distribution profile in the body, thereby, minimizing contact with healthy tissues (e.g. encapsulation in a liposomal formulation). Doxorubicin HCl liposome injection is doxorubicin HCI encapsulated in STEALTH liposomes for intravenous administration. Direct injection of doxorubicin into the body may lead to cardiac toxicity, but the liposomal formulation helps to get doxorubicin delivered to the tumour site and away from the heart muscles. Targeting drug delivery also implies presentation of a drug to a particular region of body where it is better absorbed. For example, some drugs are better absorbed in stomach and upper part of small intestine (e.g. floating drug delivery system). Another level of sophistication for targeted delivery is the intracellular localization of a drug (i.e., targeting at the cell surface, in the cytoplasm or in the nucleus).

Table 1.3: Some Examples of New Drug Delivery Systems

Therapeutic Systems	Main Features
Oral: Oral osmotic system (OROS)	Osmotic pressure is used to release the drug. A core tablet (drug + osmotic agent) is surrounded by semi permeable coating having 0.4mm diameter for the drug exit.
Hydrodynamic pressure controlled system (OROS push-pull)	It has two compartments (one contains drug and other osmotic agent) that are surrounded by semi permeable membrane. The hydrodynamic pressure releases the drug at a zero order.
Multidirectional osmotic drug absorption system (MODAS)	Similar to osmotic system designed for soluble drugs, admitting moisture and delivering soluble drug back through the same membrane.
Polymer coated pellets	The release rate of drug from coated pellets depends upon the solubility and thickness of coating.
Transdermal: Device and skin controlled	These are designed to support the passage of drug from skin surface (applied as patch) through skin layers into systemic circulation.
Ocular: Ocusert	It is elliptical flexible wafer, multilayered system consisting of drug as core surrounded by rate controlled membrane polymer. One pilocarpine ocusert provide relief for 7 days.
Lacrisert	Rod-shaped device made form hydroxy propyl cellulose used in the treatment of dry eye syndrome as an alternative to artificial tears.
Implant: Silicon polymer Matrix system	This is sterile, flexible, closed capsule of polymer surgically placed subdermally under the skin, which provide long term (up to 5 years) release of active ingredient (e.g.Levonorgestrel).
Prodrugs: Oral	It is chemically modified inert drug precursor where duration of drug action can be modified by controlled absorption of prodrugs and subsequent conversion of active drug in the blood.

Table 1.4: Examples of Targeted Drug Delivery Systems

Floating drug delivery system (FDDS)	It is based on gastric retentive system (GRS), aimed to prolong release and restrict the region of delivery to stomach. Drugs like cyclosporine, ciprofloxacin are better absorbed in stomach and upper small intestine can be made as FDDS to improve their availability.
Bio (Muco) adhesive system	It is used to localize a delivery system to a specific area for local action or increase contact time at the absorption site.
Targeted delivery through colloidal carriers (liposomes, nano-particles, resealed erythrocytes, etc)	Drug is delivered to selective site of action of organ at the predetermined rate thereby eliminating wasteful distribution of drug and subsequent reduction of adverse effects.
Drug delivery through external pump	It consists of reservoir of drug whose flow in the body is actuated to release the drug at a zero order by powered devices (i.e. pimps and implantable infusion system).

Until recently, the development of a new drug was associated with synthesis of a new chemical compound. Although, invention of new drug is being continued to win over diseases, albeit development of new techniques of drug delivery is receiving more attention than ever before. Pharmaceutical researchers are interested in new, noninvasive methods of drug delivery that aim to increase drug efficiency and safety.

The goal of optimal therapy is to deliver the drug to produce maximum simultaneous safety, effectiveness and reliability. All these rely on establishing technology of innovative devices that meets the need for effective delivery of drug to the site of action.

Of the various routes of drug administration, the oral route remains supreme, as it is the most convenient and extensively used route of administrating of drugs. It has the most patient acceptability as it provides for ease for administration. Accordingly, over the years, oral dosage forms have become increasingly sophisticated with major role being played by controlled drug delivery systems, release the drug at a predetermined rate as determined by drug's pharmacokinetics and desired concentration

The de novo design of an oral controlled drug delivery system should be primarily aimed at achieving more predictable and increased bioavailability of the drugs. However, the development process is restricted by several physiological difficulties such as inability to restrain and localize the drug delivery system within the desired region of the gastro intestinal tract and the highly variable nature of emptying process. It can be anticipated that, depending upon the physiological state of the subject and the design of pharmaceutical formulations, the empting process of in turn can lead to unpredictable bioavailability and times to achieve peak plasma levels, since the majority of drugs are preferentially absorbed in the upper part of the small intestine. Thus placement of a drug delivery system in a specific region of the GI tract and control of drug offer numerous advantages.

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and to maintain, the desired drug concentration. The idealized objective points to the two aspects most important to drug delivery, namely, spatial placement and temporal delivery of a drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. An approximately designed controlled release drug delivery system can be major advance towards solving these two problems.

GASTRORETENTIVE DRUG DELIVERY SYSTEMS

In an attempt to retain the dosage form for a prolonged period, gastro retentive system has been developed for the last two decades and is a topic of interest in terms of their potential for the controlled drug delivery at the targeted site.

Davis firstly described the concept of floating drug delivery system (FDDS). Gastric emptying of dosage forms is an extremely variable process and the ability to prolong and control the emptying time is a valuable asset for dosage forms[7], which residue in the stomach for a longer period of time than conventional dosage forms.

Gastro retentive system can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability reduces drug waste and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestine; gastro retention helps to promote better availability of new products with new therapeutic possibilities and substantial benefits for patients.

The controlled gastric retention of solid dosage forms may be achieved by the mechanism of mucoadhesion, floatation, expansion or by the simultaneous administration of pharmacological agents that delay gastric emptying.

- **Concept of Absorption Window**

Not all drugs candidates get uniformly absorbed through the G.I tract. Drugs exhibiting absorption from only a particular part of G.I tract (or) showing difference in absorption from various region of G.I tract are said to have regional variability in intestine absorption. Such drugs show absorption window, which signifies the region of G.I tract from where absorption primarily occurs. The absorption window is observed due to following factors.

1. Physio chemical factors:
    A. pH dependent solubility
    B. pH dependent stability
    C. Enzymatic degradation
2. Physiological factors:
    A. Mechanism of absorption
    B. Microbial degradation
3. Biochemical factors:

A. Intestinal metabolic enzymes (phase 1 drug metabolizing enzyme), cytochrome p450 (CYP3)

B. The multidrug efflux pump, p glycoprotein (pgp) present in the villus tip of enterocytes in the G.I.T.

- **Basic Anatomy and Physiology of Gastrointestinal Tract.**

The stomach is situated in the left upper part of the abdominal cavity immediately under the diaphragm. The stomach is divided into 3 regions.

a. Fundus, above opening of the stomach

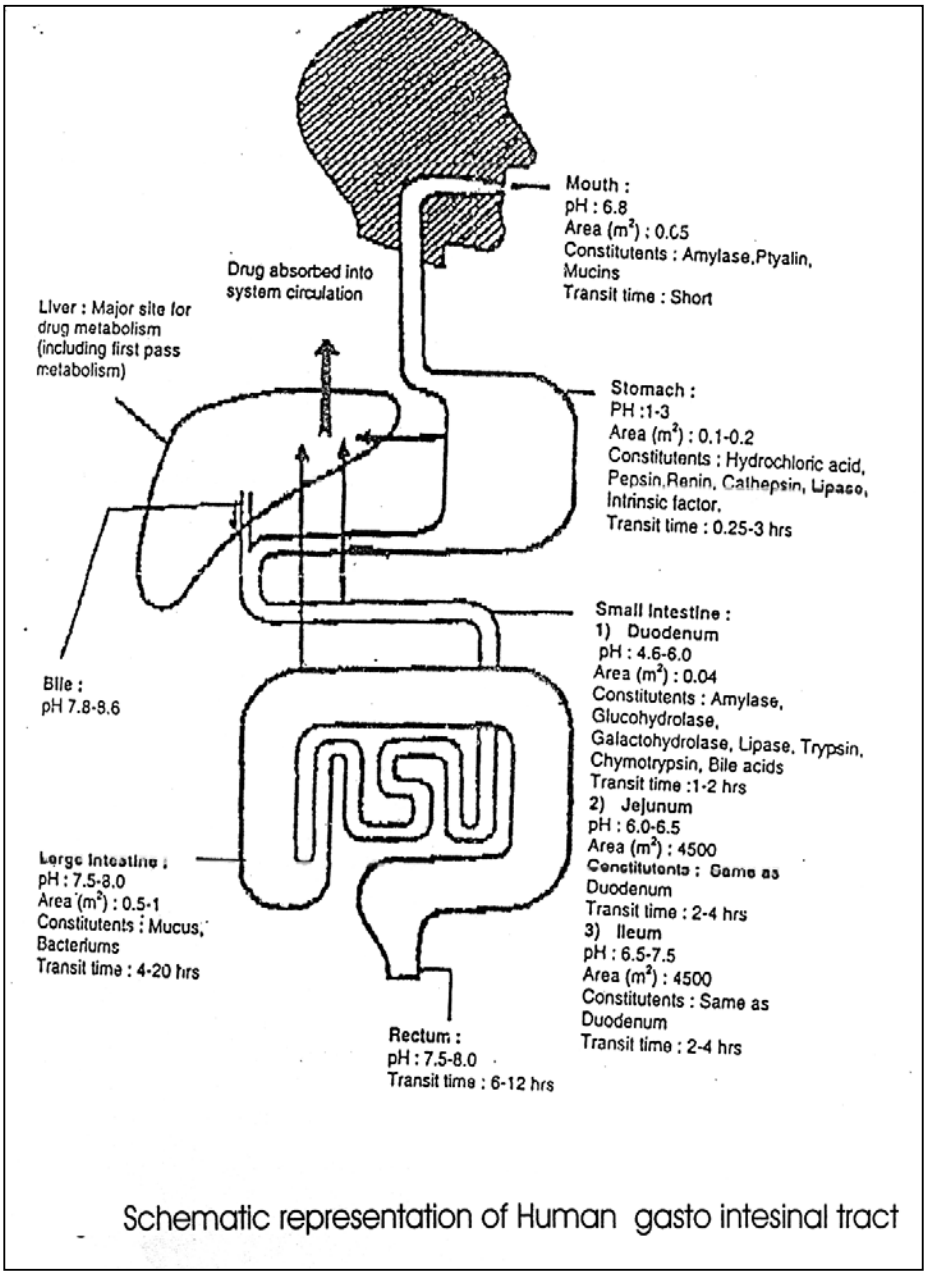
b. Body, central part

c. Antrum, pylorus

The fundus and body regions are capable of displaying a large expansion to accommodate food without much increase in the intragastric pressure. The stomach lining is devoid of villi but consists of a considerable number of gastric pits that contribute to the storage capacity of the stomach. The antrum region is known to be responsible for the mixing and grinding of gastric contents. Under fasting conditions the stomach is a collapsed bag with a residual volume of 50ml and contains a small amount of gastric fluid (pH 1-3) and air. There are two main secretions, mucus and acid, produced by specialized cells in the stomach lining. Mucus in secreted by goblet cells and gastric acid by oxyntic (parietal) cells.

The contents of the stomach are emptied through the pylorus into the proximal duodenal region of the small intestine. In humans the gastro duodenal junction controls the unidirectional passage from the stomach to the duodenum.The small intestine is a tubular viscous organ and has enormous numbers of villi on its mucosal surface that create a huge surface area (4500 m^2 compared to only 0.1-0.2 m^2 for the stomach). These villi are minute fingerlike projections of the mucosa and have a length of 0.5 – 1.5 mm.Villi are most numerous in the duodenum and proximal jejunum. The proximal small intestine is the region with the most efficient absorption. For maximal systemic bioavailability the drug should be targeted for delivery in the vicinity of this region. The colon lacks villi, and its primary function is to store indigestible food residue. It also contains a variety of floras that are normal residents of the G.I tract and may degrade the contents of the colon[4].

Fig no: 1.1



Schematic representation of Human gasto intesinal tract

- **Gastric Motility And Emptying Of Food From Stomach**

The motility of the stomach is mostly contractile, which causes grinding of food into smaller particles, mixing with gastric juice, forward and backward moment of gastric content and emptying with all action together[8]. There are two distinct modes of GI motility and secretory patterns in humans and animals, in fasted and fed

state. Fasted state is associated with various cyclic events regulating the GI motility patterns, commonly called as the Migrating Motor complex (MMC). The MMC is organized into alternating cycles of activity and quiescence and can be subdivided into basal, preburst and burst intervals, also named as phases I, II and III, respectively.

Phase – I (Basal phase):

The quiescent period lasts from 30 – 60 minutes and is characterized by lack of any secretory and electrical activity and contractile motions.

Phase II (Preburst phase):

Exhibits intermittent action potential for 20 - 40 minutes with increasing contractile motions. Bile enters the duodenum during this phase while the gastric mucus discharge occurs during the later part of phase II and throughout phase III.

Phase III (Burst phase):

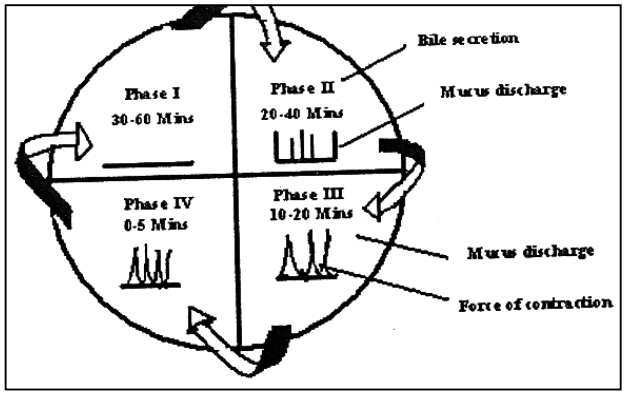
Shows the prevalence of intense large and regular contractions that sweep off the undigested food. These are also called 'housekeeper waves' and propagate for 10 - 20 minutes.

Phase IV:

It is the transition period of 0 - 5 minutes between phase III and phase IV. These interdigestive series of electrical events originate in the foregut and propagate to terminal ileum in the fasted state and repeat cyclically every -3 hour. Feeding results in the origination of a continuous pattern of spike potentials and contractions called the postprandial motility. These phases play an influential role in the performance of peroral CRDDS and GRDDS as governed by the prevalence of a particular phase during the dosage from administration.

Gastric emptying occurs during fasting as well as fed states. Like the motility pattern, the patterns of GI transit depend on whether the person is in a fasted or fed state. In addition, the physical state of the drug delivery system, either a solid or a liquid, also influences the transit time through the GI tract.

Fig no: 1.2



Fasted state:

The gastric emptying of liquids in the fasted state is a function of the volume administered. For a small volume (<100 ml), this is controlled by the existing phasic activity and liquids are emptied at the onset of phase II; most of them are gone before the arrival of phase III. For volumes larger than 150ml, liquids are emptied by characteristic discharge kinetics irrespective of phasic activity.

The fasted state-emptying pattern of liquids is independent of the presence of any indigestible solids in the stomach. Indigestible solids are emptied from the stomach as a function of their physical size. Solids of small particle size (1< mm) can be emptied with the liquid ; solids of 2 mm or greater do not empty until the arrival of phase III activity, at which time they are emptied as a bolus.

Fed state:

Following feeding the fundus of the stomach expands to accommodate food without an appreciable increase in the intragastric pressure[9]. Once in the stomach food begin emptying almost immediately. Liquids are emptied at a rate faster than that of solids, and the rate is controlled by feedback mechanisms from the duodenum and ileum. Solids are not emptied in the fed state unless they have been ground to a particular size of 2 mm or less.

Gastric secretion also starts following the ingestion of food, and its volume depends upon the nature and volume of the ingested food. The volume emptied is replaced by gastric secretion, and thus the gastric volume may actually remain

constant during the first hour of gastric emptying. The total time for gastric emptying varies in the range of 2 - 6 h.

Studies of the GI transit of dosage forms, such as tablets, capsules, and particles, have demonstrated a transit pattern similar to that of nutrients. Most dosage forms taken orally in the fasted state empty within 90 min. In the fed state non disintegrating tablets and capsules stay in the stomach for 2 - 6 h and are discharged only at the onset of the fasted state. However, disintegrating dosage forms and small particles are emptied together with the food[5]. In all instances the transit time for the small intestine is 3 - 4 h. In summary, the total transit time of foods and dosage forms in humans from stomach to the ileocecal junction is approximately 3 – 6 h in the fasted state and 6 - 10 h in the fed state.

- **Gastroretentive Technologies**

A number of techniques have been used to increase the GRT of dosage forms. These systems have been classified[10] according to their basic principle of gastric retention.

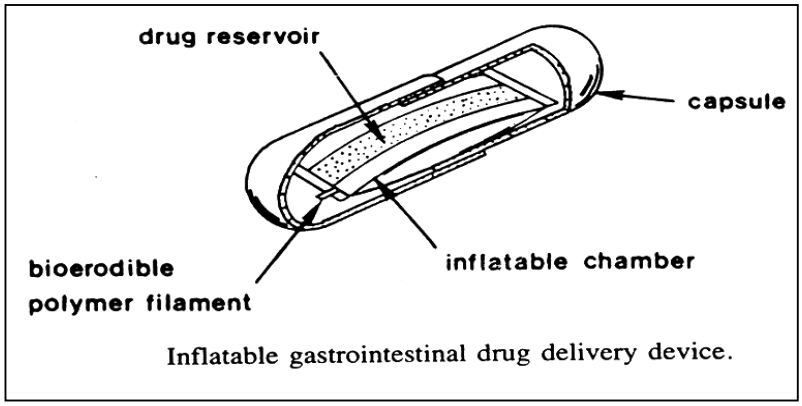
- **Floating drug delivery system**
  - ◊ Effervescent
    - ♦ Volatile liquid containing systems
    - ♦ Gas generating systems
  - ◊ Non effervescent
    - ♦ Colloidal gel barrier systems
    - ♦ Microporous compartment system
    - ♦ Alginate beads
    - ♦ Hollow microspheres
- **Swelling system**
- **Bio / Mucoadhesive system.**
- **High-density system.**

FLOATING SYSTEMS

A. Effervescent Systems.

A drug delivery system can be made to float the stomach by incorporating chamber, which may be filled with vacuum, air or inert gas. The gas in the floating chamber can be introduced either by the volatilization of an organic solvent or by the effervescent reaction between organic acids and bicarbonate salts.

Fig no: 1.3



Inflatable gastrointestinal drug delivery device.

1. Volatile liquid containing systems:

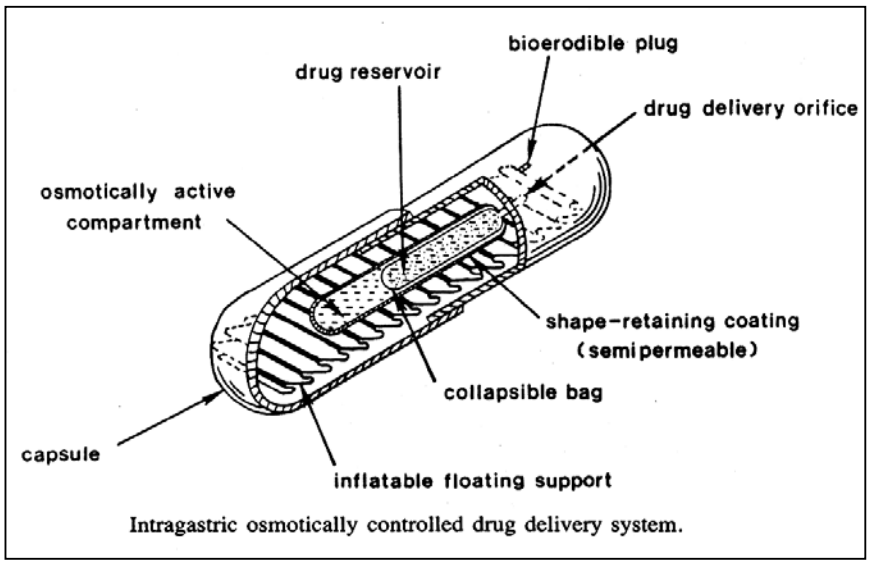
The gastric retention of a drug delivery system can be sustained by incorporating an inflatable chamber, which contains a liquid e.g. ether, cyclopentane, that gasifies at body temperature to cause the inflation of the chamber in the stomach. These devices are osmotically controlled floating systems containing a hollow deformable unit that can convert from a collapsed to an expanded position, and returns to collapsed position after an extended period. The deformable systems consists of two chambers separated by an impermeable, pressure responsive, movable bladder. The first chamber contains the drug and the second chamber contains the volatile liquid. The device inflates, and the drug is continuously released from the reservoir into the gastric fluid. The device may also consist of a bioerodible plug made up of from the reservoir into the gastric fluid. The device may also consist of a bioerodible plug made up of PVA, polyethylene, etc, that gradually dissolves causing the inflatable chamber to release gas and collapse after a redetermined time to permit the spontaneous ejection of the inflatable system from the stomach.

2. Gas generating systems.

These buoyant delivery systems utilize effervescent reaction between carbonate (or) bicarbonate salts and citric (or) tartaric acid to liberate carbon dioxide, which gets entrapped in the jellified hydrocolloid layer of the system, thus decreasing its specific gravity and making it float over chime. These tablets may be either single layered wherein the carbon dioxide generating components are intimately mixed within the tablet matrix, or they may be bilayered in which the gas generating components are compressed in one hydrocolloid containing layer, and the drug in other layer formulation for a sustained release effect.

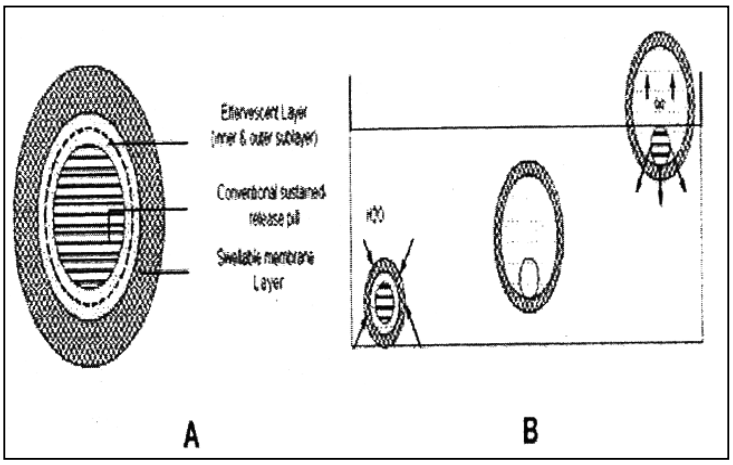
Multi – unit types of floating pills, which generate carbon dioxide, have also been developed. The system consists of a sustain release pill as seed, surrounded by double layers. The inner layer is an effervescent layer containing sodium bicarbonate and tartaric acid. The outer layer is of a swellable membrane layer containing PVA, shellac, etc. Effervescent layer is divided into two sub layers to avoid direct contact between sodium bicarbonate and tartaric acid. When the system is immersed in buffer solution at 37ºC swollen pills, like balloons are formed having density less than 1 g/ml. This occurs due to the carbon dioxide formation by neutralization of the inner effervescent layer with the diffusion of water through the outer swellable membrane layer. These kinds of systems float completely within 10 minutes, and remain floating over extended periods of 5-6 hours.

Fig no: 1.4



Intragastric osmotically controlled drug delivery system.

Fig no: 1.5



B. Non Effervescent System

1. Colloidal Gel Barrier System

Hydrodynamically Balanced System[11] was first designed by Sheth & Tosounian[8] in 1975. Such systems contain drugs with gel forming hydrocolloids meant to remain buoyant on the stomach contents. This prolongs GI residence time and maximizes drug reaching its absorption site in the solution form and hence is ready for absorption.

These systems incorporate a high level (20 - 75% w/w) of one or more gel – forming, highly swellable, cellulose – type hydrocolloids (e.g. hydroxyl ethyl cellulose (HEC), hydroxy propyl cellulose (HPC), hydroxyl propyl methylcellulose (HPMC), sodium carboxy methyl cellulose(NaCMC) polysaccharides and matrix forming polymers such as polycarbophil, polyacrylates and polystyrene, incorporated either in tablets or in capsules.

On coming in contact with gastric fluid, the hydrocolloid in the system hydrates and forms a colloidal gel barrier around its surface.

This gel barrier controls the rate of fluid penetration into the device and consequent release of the drug. As the exterior surface of the dosage form goes into the solution, the gel layer is maintained by the adjacent hydrocolloid layer becoming

hydrated. The air trapped in by the swollen polymer maintains a density less than unity and confers buoyancy to these dosage forms.

Fig no: 1.6

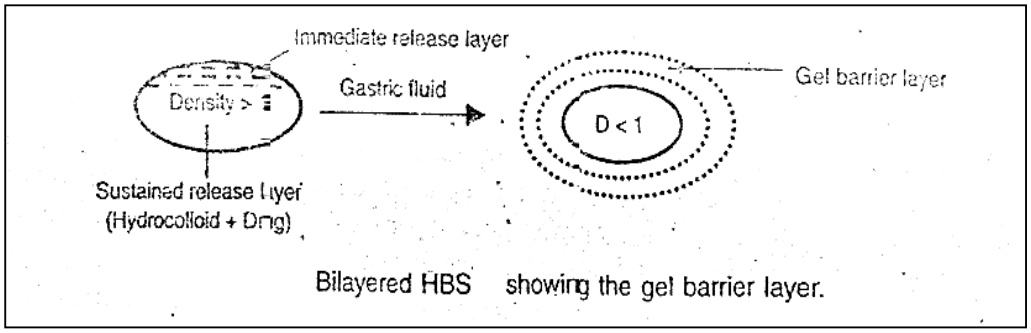
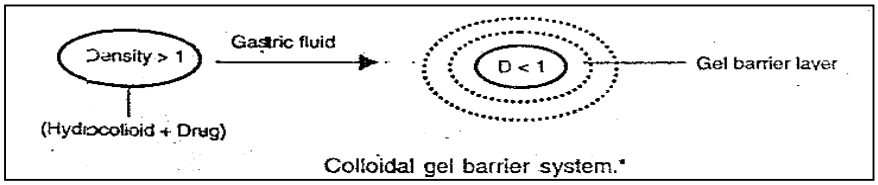


Fig no: 1.7



2. Microporous Compartment system:

This technology is based on the encapsulation of a drug reservoir inside a microporous compartment with apertures along its top and bottom walls. The peripheral walls of the drug reservoir compartment are completely sealed to prevent any direct contact of the gastric mucosal surface with the undissolved drug. In stomach, the flotation chamber containing entrapped air causes the delivery system to float over the gastric contents. Gastric fluid enters through the apertures, dissolves the drug, and carries the dissolved drug for continuous transport across the intestine for absorption.

3. Alginate beads

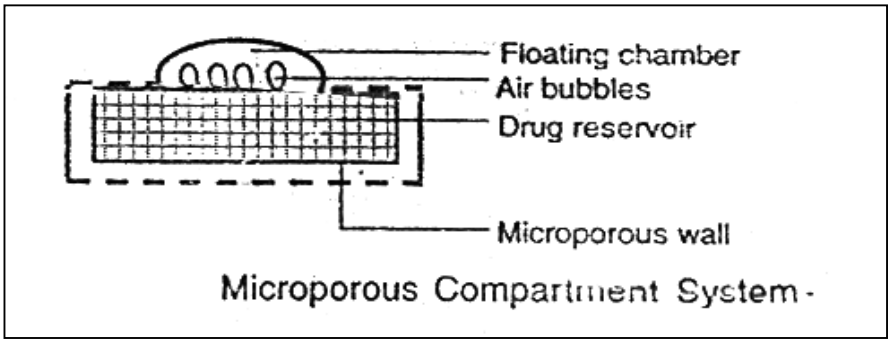
Multiunit floating dosage forms developed from freeze dried calcium alginate spherical beads of approximately 2.5 mm in diameter can be prepared by

dropping a sodium alginate solution into aqueous solution of calcium chloride, causing precipitation of calcium alginate. The beads are then separated; snap frozen in liquid nitrogen, and freeze dried at 40^0 for 24 h, leading to the formation of a porous system, which can maintain a floating force for over 12 h.

When compared with solid beads, which had a short residence, time of 1 h, these floating beads had a prolonged residence time of more than 5.5 h.

Floating system comprising of a calcium alginate core separated by an air compartment from a membrane of calcium alginate or calcium alginate / polyvinyl alcohol (PVA) have also been developed The porous structure generated by leaching of PVA, (a water – soluble additive in coating composition) was found to increase the membrane permeability, preventing the collapse of air compartment.

Fig no: 1.8



BIO / MUCO ADHESIVE SYSTEMS

Bio / Muco adhesive systems are those which bind to the gastric epithelial cell surface or mucin and serve as a potential means of extending the gastric retention of drug delivery system (DDS) in the stomach, by increasing the intimacy and duration of contact of drug with the biological membrane. The concept is based on self-protecting mechanism of GI tract.

SWELLING SYSTEMS

These are the dosage forms, which after swallowing; swell to an extent that prevents their exit from the pylorus. As a result, the dosage form is retained in the stomach for a long period of time. These systems may be named as "plug type systems" since they exhibit the tendency to remain lodged at the pyloric sphincter. The formulation is designed for gastric retention and controlled delivery of the drug into the gastric cavity. Such polymeric matrices remain in the gastric cavity for several hours even in the fed state. Sustained and controlled drug release may be achieved by selection of proper molecular weight polymer, and swelling of the polymer retards the drug release.

HIGH DENSITY SYSTEMS

High – density system is another modification of GRDDS. These systems with a density of about 3 g/cm^3 are retained in the rugae of the stomach and are capable of withstanding its peristaltic movements. The density of 2.6 – 2.8 g/cm^3 acts as a threshold density after which such systems can be retained in the lower part of the stomach. Such a phenomenon needs to be confirmed by clinical studies after which the heavy pellet formulations can hit the market in near future.

1.5 Pharmaceutical Aspects of Expandable Gastro Retentive Dosage Forms (GRDF)

In designing gastro retentive dosage the following characteristic[12] should be sought.

a) Retention in the stomach according to the clinical demand

b) Convenient intake

c) Ability to load substantial amount of drug with different physicochemical properties and release them in a controlled manner

d) Complete degradation

e) No effect on gastric motility including emptying pattern

f) No other local adverse effects on the gastro intestinal wall

Factors Affecting the Performance of GRDDS

The performance of GRDDS is affected by various factors, as described below:

1. Formulation Factors (Density, Shape and Size of Dosage Form)

FDDS are retained in the stomach by virtue of their floating tendency for which their density should be less than that of the gastric contents. The effect of various geometric shapes on gastric retention of the dosage form has been well studied. Six shapes (ring, tetrahedron, clove leaf, string, pellet, and disc) were screened *in vivo* for their gastric retention potential. The tetrahedrons (each leg 2 cm long) and rings (3.6 cm in diameter) exhibited nearly 100% retention at 24 hours. On the other hand clove leaves (2.2. – 3.2 diameter) exhibited 40-67%, discs (2.5 cm diameter) 67%, string (12 cm x 2mm x 2 mm / 24cm x 2mm x 2mm) 0%, and pellet (4mm) 0% retention at 24 hours.

Size of the dosage form can also be a controlling factor behind its gastric emptying. Small – sized tablets are emptied from the stomach during the digestive phase, while the larger size tablets are expelled during the housekeeping waves.

Varying gastric emptying times were observed for non-disintegrating tablets of different sizes. Longest gastric emptying time was observed for 13mm tablets (171 ±13 min), followed by 11 mm (128 ± 17 mm) and 7mm (116 ± 19 min) tablets. These results are in accordance with the aperture of resting pylorus, 12.8 + 7 mm. This can be taken as the critical value for GI transit of different size dosage forms.

2. Idiosyncratic Factors

a. Concomitant intake of food and drugs like anticholinergics (e.g. atropine, propantheline), opiates (codeine) and prokinetic agents (metoclopramide, cisapride) etc.

b. Biological factors like gender, age, posture, body mass index and disease state (e.g. diabetes, Crohn's disease).

Presence of food is known to modify the gastric retention of the dosage form. Gastric retention increases in the presence of food, leading to the increase in the dissolution of the drug and longer residence of the dosage form at the most favorable

sites of absorption. Further, the nature, caloric content and the frequency of intake of food affect the gastric retention of the dosage form.

Moreover co administration of GIT motility decreasing drugs can increase the gastric emptying time, on the contrary, these drugs should be contraindicated with Mucoadhesive systems as they reduce the gastric secretion and induce the drying of mucus membrane.

Women and elderly show a slower gastric emptying than men. Intrasubject and intersubject variations are also observed in the gastric and intestinal transit times. Subject posture (standing and supine) also leads to variable intragastric behaviors. The upright position protects the floating form against postprandial emptying, as the floating form remains continuously above the gastric contents irrespective of their size.

Evaluation of drugs product is a tool to ensure.

(I) Performance characteristics and

(II) Control batch –to-batch quality

Apart from routine tests like general appearance, hardness, friability, drug content, weight variation, drug release etc., GRDDS need to be evaluated for gastro retentive performance by carrying out specific tests

3. *In vitro* and *In vivo* Evaluation of Floating Systems

1. Floating time

The test for buoyancy is usually performed in simulated gastric and intestinal fluids maintained at 37°C. The floating time is determined by the USP dissolution apparatus containing 900 ml of 0.1 N HCl as the testing medium maintained at 37°C. Time required for dosage to float was termed as buoyancy lag time.

2. Gastro retentive: R-Scintigraphy

In vivo visualization is a crucial parameter for evaluating the GI tract retention characteristics of the dosage form. The inclusion of a radio material into a solid dosage form enables it to the x-rays, similarly, the inclusion of a γ emitting radionuclide in a formulation. The use of X-rays involves exposing a patient to an X-beam, thus aiding in the visualizing of the GI tract transit of dosage from. In case

of γ scintigraphy; the γ rays emitted by the radionuclide are focused on a camera, which helps to monitor the location of the dosage from in the GI tract.

3. Radiology

In this method, radio opaque markers may be applied to study the effects of GRDF on experimental models of this evaluation on gastric emptying.

4. Gastroscopy

It is used to inspect visually the effects of prolonged stay in the stomach *milleu* on the gastro retentive floating system.

5. Magnetic marker monitoring

It yields data regarding the location of dosage from along the GI tract by means of magnetic marker dosage form.

Table 1.5. Marketed Preparations of Floating Drug Delivery Systems

S. no	Product	Active Ingredient
1	Madopar	Levodopa and benserzide
2	Valrelease	Diazepam
3	Topalkan	Aluminum magnesium antacid[13]
4	Amalgate float coat	Antacid[14]
5	Liquid gavison	Alginic acid and sodium bicarbonate[15]
6	Cifran OD	Ciprofloxacin

Table 1.6. List of Drugs Formulated as Single and Multiple Unit Forms of Floating Drug Delivery Systems.

Tablets	Chlorpheniramine maleate
	Theophylline
	Furosemide
	Ciprofolxacin
	Pentoxyfillin
	Captopril
	Acetylsalicylic acid
	Nimodipine
	Amoxycillin trihydrate
	Verapamil HCl
	Isosorbide di nitrate
	Sotalol
	Atenolol
	Isosorbide mono nitrate[16]
	Acetaminophens[18]
	Ampicillin[19]
	Cinnarazine[20]
	Diltiazem[21]
	Florouracil[22]
	Piretanide[23]
	Prednisolone[24]
	Riboflavin- 5′ Phosphate[25]
Capsules	Nicardipine
	$_{L}$- Dopa and benserazide
	Chlordiazepoxide HCl[17]
	Furosemide
	Misoprostal
	Diazepam[26]
	Propranlol[27]
	Urodeoxycholic acid[28]
Microspheres	Verapamil
	Aspirin, griseofulvin, and p-nitroaniline
	Ketoprofen
	Tranilast
	Iboprufen
	Terfenadine[29]
Granules	Indomethacin
	Diclofenac sodium
	Prednisolone
Films	Drug delivery device
	Cinnarizine
Powders	Several basic drugs[30]

Applications of Floating Drug Delivery Systems

Floating drug delivery systems offers several applications for drugs having poor bioavailability because of narrow absorption window in the upper part of the gastro intestinal tract. It retains the dosage form at the site of absorption and thus enhance the bioavailability of the drug .The main applications are summarized as follows

- **Sustained drug delivery**

HBS systems can remain in the stomach for long periods and hence can release the drug over a prolonged period of time. Recently sustained release floating capsules of nicardipine hydrochloride was developed and was evaluated in vivo. The formulation was compared with marketed conventional product (MICARD).

- **Site specific Drug delivery**

These systems are particularly advantageous for drugs that are specifially absorbed from stomach or the proximal part of the small intestine, e.g. furosemide, riboflavin.

It has been reported that a monolithic dosage form with prolonged gastric residence time was developed and the bioavailability was increased. AUC obtained with the floating tablets was 1.8 times more than that of usual conventional dosage form. A bilayered floating capsule was developed for local delivery of misosprostol, which is a synthetic analog of prostaglandin of NSAIDs. By targeting slow delivery of misosprostol to the stomach, desired therapeutic levels could be achieved and drug waste could be reduced

- **Absorption Enhancement**

Drugs that have poor bioavailability because of site specific absorption from the upper part of the gastro intestinal tract are potential candidates to be formulated as floating drug delivery system, thereby maximizing their absorption.

A significant increase in the bioavailability of floating dosage form (42.9%) could be achieved as compared with commercially available LASIX tablets (33.4%) and enteric-coated LASIX long product (29.5%).

GASTRIC CANCER

Epidemiology

In the early 1900s, the leading cause of cancer death in American males was gastric cancer; it was the third leading cause of cancer death in females at this time. Since them, the incidence of gastric cancer in the United States has decreased. The estimated total number of new cases of gastric cancer in the United states for 1995 is 22,800 (14,000 new cases in men; 8,800 new cases in women). Gastric cancer worldwide is most prevalent in Asia. Other countries with a high rate of gastric cancer include the former Soviet Union, Costa Rica and South America.

Etiology

The risk factors for the development of gastric cancer[31] are believed to be associated with the environment. Diet is probably the most commonly postulated environmental factor studied in relation to gastric cancer. Diets including high concentrations of nitrates/nitrites, high salt intakes, inappropriate food storage, food spoilage/fermentation, and other factors fostering nitrosamine formation have been related to increasing the risk of developing gastric cancer. One proposed chemo preventive agent is ascorbic acid or Vitamin C. The mechanism of action of ascorbic acid is thought to be through this vitamin's ability to prevent the reduction of nitrous acid to N-nitroso compounds. These N-nitroso compounds are carcinogenic in the stomach.

Another factor that may increase the risk of gastric cancer is chromic infection with Helicobacter pylori. H. pylori are commonly present in patients with severe gastritis and chronic atrophic gastritis. It is a common infection, with approximately 50% of adults over the age of 50 in North America and virtually 100% of adults in some developing or newly industrialized countries infected. It is estimated that the incidence of gastric cancer is 6 times higher in a 100% infected population when compared with a non-infected population. Still, only a small percentage of the total number of patients infected with H. pylori will actually develop gastric cancer. Infection with H. pylori indirectly causes gastric cancer. The chronic gastritis secondary to H. pylori leads to an increase in cell turnover and

intestinal metaplasia development. Other risk factors increasing the occurrence of gastric cancer include family history, individuals from blood group A, and individuals with pernicious anemia, atrophic gastritis, prior gastric surgery, gastric polyps, or achlorhydria. Individuals from lower socioeconomic classes tend to be at increased risk for the development of gastric cancer. This increased risk is thought to be secondary to dietary and environmental factors common in this population. Smoking has also been associated with increased risk of gastric cancer. However, alcohol consumption has not been shown to increase the risk of gastric cancer.

Pathophysiology

The majority of malignant gastric cancers are adenocarcinomas, accounting for approximately 84% of all gastric neoplasms. The incidence of other, less common histologic classifications include signet ring cell tumors (8%), mucinous adenocarcinomas (3%) and diffuse type adenocarcinoma, intestinal type adenocarcinoma, papillary adenocarcinoma, undifferentiated carcinoma, adenosquamous carcinoma and tubular adenocarcinoma, each identified in less than 2% of patients.

Approximately 30% of primary gastric cancers occur in the upper third of the stomach, 14% in the middle third, and 26% in the lower third. The entire stomach may be involved in up to 10% of patients. Gastric cancer has four major patterns of spread: direct extension into the surrounding tissues and organs such as the liver, diaphragm, pancreas, spleen, biliary tract, and transverse colon; nodal metastases, both local (perigastric, celiac axis, porta hepatis, retroperitoneal) and distant (Virchow's node, left axillary nodes); hematogenous spread to liver, lung, bone, and brain; and intraperitoneal dissemination in the pelvis. Intraperitoneal spread may be evidenced by the presence of peritoneal implants or ascites.

Overall the prognosis for patients diagnosed with gastric cancer is poor, mainly because only a few patients are diagnosed with early stage disease. The overall survival is strongly correlated with stage at diagnosis. The 5-year survival rate for stage I gastric cancer is 90%; stage II, 70%; stage III, 45%; and stage IV less than 10%.

Clinical Presentation

Patients with early gastric cancer are typically men (male: female ratio 1.5:1 to 2:1) who are 44 to 70 years of age. By the time gastric cancer is diagnosed, it is usually advanced. This is primarily because the signs and symptoms of early gastric cancer are similar to those of peptic ulcer disease. The first signs and symptoms that patients may have are mild epigastric pain and dyspepsia. About 40% of patients experience nausea and vomiting. When gastric cancer is still in the early stages, weight loss is minimal, in spite of anorexia. Only one fourth of patients with early gastric cancer demonstrate signs and symptoms of upper gastrointestinal bleeding, with associated anemia. Once the disease has advanced, patients will complain of significant weight loss (>10 pounds), abdominal pain anorexia, haematemesis, guaiac positive stools and anemia. Other physical changes that might suggest advanced gastric cancer when observed in conjunction with the previous signs include palpable lymph nodes, palpable ovarian mass, hepatomegaly, palpable abdominal mass, ascites, jaundice, and cachexia.

Many patients report experiencing the symptoms of mild epigastric pain for 21 to 36 months. Patients who present with advanced gastric cancer often relate having experienced symptoms for at least the previous 6 to 8 months. In general, the abdominal pain and discomfort experienced with either early or advanced gastric cancer is not relieved by food or antacids.

Diagnosis

The differential diagnosis between peptic ulcer disease and gastric cancer must be made in these patients, since the presenting signs and symptoms are so similar. Currently, no blood test that can be used in the definitive diagnosis of gastric cancer is available. Blood tests that may be useful in determining the extent of disease include complete blood count, liver function tests (bilirubin, alkaline phosphatase, LDH, ALT, AST), and carcinoembryonic antigen (CEA). CEA is not used as a diagnostic tool, since it is elevated in only 15 to 30% of patients with advanced disease. CEA may be useful in assessment of response to treatment or evaluating recurrence after potentially curative surgical resection.

Historically, the diagnostic procedure performed on patients with upper gastrointestinal complaints has been the upper GI roentgenogram. However, over the last 10 to 15 years there has been a decrease in the use of upper GI roentgenogram and an increased use of upper GI endoscopy for the diagnosis of gastric symptomatology. The increase in the use of endoscopy demonstrates the usefulness of direct visualization of the stomach with an additional advantage of obtaining biopsy specimens. Thus esophagogastroduodenoscopy has become the diagnostic procedure of choice.

Endoscopic ultrasonography (EUS) can also be performed to diagnose gastric cancer and the extent of disease. The main utility of EUS is in preoperative staging by enabling evaluation of the depth of cancer invasion into the gastric wall. EUS has a 91% accuracy rate in evaluating depth of invasion and may also be useful in diagnosing perigastric metastatic lymph nodes.

Screening for early gastric cancer is currently being conducted in areas associated with a high risk of gastric cancer, primarily Japan, South America, and Eastern Europe. Again, esophagogastroduodenoscopy is the diagnostic procedure used in these areas. Another diagnostic test often ordered for patients with suspected advanced gastric cancer is a computed tomography scan of the abdomen. This test is performed to evaluate the presence / absence of distant metastases.

Staging and Prognosis

The staging of gastric cancer depends on the extent of the disease. The TNM classification is used to describe the stage of gastric cancer as recommended by the American Joint Committee on Cancer. The percentage of patients presenting at the various stages are stage I (1-5%), stage II (10-15%), stage III (17-20%) and stage IV (72%).

Treatment

◊ Surgical Therapy

Surgery is the only therapeutic option that offers a potential cure for the gastric cancer patient. The amount of stomach removed should be enough to allow

ample tumor-free margins, with the regional lymph nodes also being removed should be enough to allow ample tumor-free margins, with the regional lymph nodes also being removed. Extension of the surgical margins into the adjacent organs should be done only if necessary.

In order to ensure tumor-free margins, either a subtotal or total gastrectomy will be performed, based on the location of the tumor in the stomach and on the pattern of spread of the tumor within the stomach.

◊ Radiation Therapy

Radiation therapy has a limited role in the treatment of gastric cancer. This is primarily due to the difficulty in delivering the required dose in the stomach area. There are many normal tissues in this area that are highly radiosensitive: the spinal cord, kidneys, liver, and small intestines. The use of intraoperative radiation therapy (IORT) is one appealing route of radiation delivery in this patient population. This method allows delivery of the required doses of radiation directly to the tumor and does not exceed the tolerance level of the normal tissues.

◊ Neoadjuvant Chemotherapy

The goal of neoadjuvant chemotherapy is to improve resectability of tumors in patients with locally advanced disease by decreasing the tumor burden and therefore, increase the survival time.

◊ Adjuvant Chemotherapy

Many trials with adjuvant chemotherapy regimens have been made in an effort to improve survival rates in resected gastric cancer patients. These adjuvant regimens have been compared to surgery alone. Single agents that have been used as adjuvant chemotherapy in separate trials are thiotepa, floxuridine, and high-dose mitomycin C. Only mitomycin C demonstrated an increase in survival when compared to surgery alone.

The Gastrointestinal Tumor Study Group (GITSG) reported a survival benefit in the group of patients receiving adjuvant chemotherapy with 5-fluorouracil

and methyl-CCNU. 5-fluorouracil, doxorubicin, and mitomycin C (FAM) is a regimen that has been tested extensively in the treatment of advanced disease.

◊ Chemotherapy of Advanced disease

Numerous single agents have been tested in the treatment of advanced gastric cancer. Of these agents tested 5-fluorouracil, doxorubicin, mitomycin C and cisplatin have demonstrate activity, investigators have combined these agents in a number of varying regimens. One of the first combinations used in advanced gastric cancer was 5-fluorouracil doxorubicin, mitomycin, also known as the FAM regimen.

Numerous other combination regimens have been compared to FAM. All regimens produced a response rate that was comparable to FAM with no difference in survival. The North Central Cancer Treatment Group (NCCTG) concluded after studying FAM vs. single-agent 5-fluorouracil that 5-FU should be considered the standard treatment for advanced gastric cancer, since less expense and toxicities were observed with comparable response rates. A recent four-arm study by the NCCTG compared 5-fluorouracil, doxorubicin, and methyl CCNU (FAMe); 5-fluorouracil, doxorubicin, and cisplatin (FAP); and FAMe alternating with triazinate to single agent 5-FU. Once again, single-agent 5-FU was less toxic than any of the combination regimens and the combination regimens did not demonstrate a survival advantage over the single agent.

Thus, new agents are continuously being screened for the treatment of advanced gastric cancer. Outside of a clinical trial the least expensive and least toxic chemotherapy regimens should be used in the treatment of advanced gastric cancer.

Since the signs and symptoms of early gastric cancer are so similar to those of peptic ulcer disease, gastric cancer is usually diagnosed in advanced stages. The treatment of gastric cancer depends on the stage of disease. As with other cancers of the gastrointestinal tract, surgery is the only means to achieve a cure. Patients who are able to undergo surgery for curative purposes are those with early stage disease (stage I and II). In patients with locally advanced or metastatic disease, surgery is only performed as a palliative therapy.

The role of neoadjuvant or adjuvant chemotherapy with or without radiation therapy needs to be explored further, as conflicting reports currently exist. Multiple combination chemotherapy regimens have been used in the treatment of unresectable gastric cancer, each providing the patient with varying response rates and toxicities. Unfortunately, none of the regimens provides the patient with an improved survival. Because of this, new agents and dosage forms continue to be studied in the treatment of gastric cancer.

LITERATURE REVIEW

- Timmermans and Andre[32] studied the effect of size of floating and nonfloating dosage forms on gastric emptying and concluded that the floating units remained buoyant on gastric fluids. These are less likely to be expelled from the stomach compared with the nonfloating units, which lie in the antrum region and are propelled by the peristaltic waves.
- It has been demonstrated using radiolabeled technique that there is a difference between gastric emptying times of a liquid, digestible solid, and indigestible solid. It was suggested that the emptying of large (>1 mm) indigestible objects from stomach was dependent upon interdigestive migrating myoelectric complex. When liquid and digestible solids are present in the stomach, it contracts ~3 to 4 times per minute leading to the movement of the contents through partially opened pylorus. Indigestible solids larger than the pyloric opening are propelled back and several phases of myoelectric activity take place when the pyloric opening increases in size during the housekeeping wave and allows the sweeping of the indigestible solids. Studies have shown that the gastric residence time (GRT) can be significantly increased under the fed conditions since the MMC is delayed. [33]
- Several formulation parameters can affect the gastric residence time. More reliable gastric emptying patterns are observed for multiparticulate formulations as compared with single unit formulations, which suffer from "all or none concept." As the units of multiparticulate systems are distributed freely throughout the gastrointestinal tract, their transport is affected to a lesser extent by the transit time of food compared with single unit formulation.[34]
- Size and shape of dosage unit also affect the gastric emptying. Timmermans[35] reported that tetrahedron- and ring-shaped devices have a better gastric residence time as compared with other shapes. The diameter of the dosage unit is also equally important as a formulation parameter. Dosage forms having a diameter of more than 7.5 mm show a better gastric residence time compared with one having 9.9 mm.

The density of a dosage form also affects the gastric emptying rate. A buoyant dosage form having a density of less than that of the gastric fluids floats. Since it is away from the pyloric sphincter, the dosage unit is retained in the stomach for a prolonged period.

- Timmermans et al studied the effect of buoyancy, posture, and nature of meals on the gastric emptying process in vivo using gamma scintigraphy.[36] To perform these studies, floating and nonfloating capsules of 3 different sizes having a diameter of 4.8 mm (small units), 7.5 mm (medium units), and 9.9 mm (large units), were formulated. On comparison of floating and nonfloating dosage units, it was concluded that regardless of their sizes the floating dosage units remained buoyant on the gastric contents throughout their residence in the gastrointestinal tract, while the nonfloating dosage units sank and remained in the lower part of the stomach. Floating units away from the gastro-duodenal junction were protected from the peristaltic waves during digestive phase while the nonfloating forms stayed close to the pylorus and were subjected to propelling and retropelling waves of the digestive phase. It was also observed that of the floating and nonfloating units, the floating units were had a longer gastric residence time for small and medium units while no significant difference was seen between the 2 types of large unit dosage forms.

  These are matrix types of systems prepared with the help of swellable polymers such as methylcellulose and chitosan and various effervescent compounds, eg, sodium bicarbonate, tartaric acid, and citric acid. They are formulated in such a way that when in contact with the acidic gastric contents, CO_2 is liberated and gets entrapped in swollen hydrocolloids, which provides buoyancy to the dosage forms.

- Ichikawa et al[37] developed a new multiple type of floating dosage system composed of effervescent layers and swellable membrane layers coated on sustained release pills. The inner layer of effervescent agents containing sodium bicarbonate and tartaric acid was divided into 2 sublayers to avoid direct contact between the 2 agents. These sublayers were surrounded by a swellable polymer membrane containing polyvinyl acetate and purified shellac. When this system

was immersed in the buffer at 37ºC, it settled down and the solution permeated into the effervescent layer through the outer swellable membrane. CO_2 was generated by the neutralization reaction between the 2 effervescent agents, producing swollen pills (like balloons) with a density less than 1.0 g/mL. It was found that the system had good floating ability independent of pH and viscosity and the drug (para-amino benzoic acid) released in a sustained manner.

- Ichikawa et al[38] developed floating capsules composed of a plurality of granules that have different residence times in the stomach and consist of an inner foamable layer of gas-generating agents. This layer was further divided into 2 sublayers, the outer containing sodium bicarbonate and the inner containing tartaric acid. This layer was surrounded by an expansive polymeric film (composed of poly vinyl acetate [PVA] and shellac), which allowed gastric juice to pass through, and was found to swell by foam produced by the action between the gastric juices and the gas-generating agents. It was shown that the swellable membrane layer played an important role in maintaining the buoyancy of the pills for an extended period of time. Two parameters were evaluated: the time for the pills to be floating (TPF) and rate of pills floating at 5 hours (FP_{5h}). It was observed that both the TPF and FP_{5h} increased as the percentage of swellable membrane layer coated on pills having a effervescent layer increased. As the percentage of swellable layer was increased from 13% to 25% (wt/wt), the release rate was decreased and the lag time for dissolution also increased. The percentage of swellable layer was fixed at 13% wt/wt and the optimized system showed excellent floating ability in vitro (TPF ~10 minutes and FP_{5h} ~80%) independent of pH and viscosity of the medium.
- Yang et al[39] developed a swellable asymmetric triple-layer tablet with floating ability to prolong the gastric residence time of triple drug regimen (tetracycline, metronidazole, and clarithromycin) in Helicobacter pylori–associated peptic ulcers using hydroxy propyl methyl cellulose (HPMC) and poly (ethylene oxide) (PEO) as the rate-controlling polymeric membrane excipients. The design of the delivery system was based on the swellable asymmetric triple-layer tablet approach. Hydroxypropylmethylcellulose and poly (ethylene oxide) were the

major rate-controlling polymeric excipients. Tetracycline and metronidazole were incorporated into the core layer of the triple-layer matrix for controlled delivery, while bismuth salt was included in one of the outer layers for instant release. The floatation was accomplished by incorporating a gas-generating layer consisting of sodium bicarbonate: calcium carbonate (1:2 ratios) along with the polymers. The in vitro results revealed that the sustained delivery of tetracycline and metronidazole over 6 to 8 hours could be achieved while the tablet remained afloat. The floating feature aided in prolonging the gastric residence time of this system to maintain high-localized concentration of tetracycline and metronidazole

- Ozdemir et al[40] developed floating bilayer tablets with controlled release for furosemide. The low solubility of the drug could be enhanced by using the kneading method, preparing a solid dispersion with β cyclodextrin mixed in a 1:1 ratio. One layer contained the polymers HPMC 4000, HPMC 100, and CMC (for the control of the drug delivery) and the drug. The second layer contained the effervescent mixture of sodium bicarbonate and citric acid. The in vitro floating studies revealed that the lesser the compression force the shorter is the time of onset of floating, ie, when the tablets were compressed at 15 MPa, these could begin to float at 20 minutes whereas at a force of 32 MPa the time was prolonged to 45 minutes. Radiographic studies on 6 healthy male volunteers revealed that floating tablets were retained in stomach for 6 hours and further blood analysis studies showed that bioavailability of these tablets was 1.8 times that of the conventional tablets. On measuring the volume of urine the peak diuretic effect seen in the conventional tablets was decreased and prolonged in the case of floating dosage form.
- Choi et al[41] prepared floating alginate beads using gas-forming agents (calcium carbonate and sodium bicarbonate) and studied the effect of CO_2 generation on the physical properties, morphology, and release rates. The study revealed that the kind and amount of gas-forming agent had a profound effect on the size, floating ability, pore structure, morphology, release rate, and mechanical strength of the floating beads. It was concluded that calcium carbonate formed smaller but stronger beads than sodium bicarbonate. Calcium carbonate was shown to be a

less-effective gas-forming agent than sodium bicarbonate but it produced superior floating beads with enhanced control of drug release rates. *In vitro* floating studies revealed that the beads free of gas-forming agents sank uniformly in the media while the beads containing gas-forming agents in proportions ranging from 5:1 to 1:1 demonstrated excellent floating (100%).

- Li et al[42, 43] evaluated the contribution of formulation variables on the floating properties of a gastro floating drug delivery system using a continuous floating monitoring device and statistical experimental design. The formulation was conceived using taguchi design. HPMC was used as a low-density polymer and citric acid was incorporated for gas generation. Analysis of variance (ANOVA) test on the results from these experimental designs demonstrated that the hydrophobic agent magnesium stearate could significantly improve the floating capacity of the delivery system. High-viscosity polymers had good effect on floating properties. The residual floating force values of the different grades of HPMC were in the order K4 M~ E4 M~K100 LV> E5 LV but different polymers with same viscosity, ie, HPMC K4M, HPMC E4M did not show any significant effect on floating property. Better floating was achieved at a higher HPMC/carbopol ratio and this result demonstrated that carbopol has a negative effect on the floating behavior.
- Penners et al[44] developed an expandable tablet containing mixture of polyvinyl lactams and polyacrylates that swell rapidly in an aqueous environment and thus reside in stomach over an extended period of time. In addition to this, gas-forming agents were incorporated. As the gas formed, the density of the system was reduced and thus the system tended to float on the gastric contents.
- Fassihi and Yang[45] developed a zero-order controlled release multilayer tablet composed of at least 2 barrier layers and 1 drug layer. All the layers were made of swellable, erodible polymers and the tablet was found to swell on contact with aqueous medium. As the tablet dissolved, the barrier layers eroded away to expose more of the drug. Gas-evolving agent was added in either of the barrier layers, which caused the tablet to float and increased the retention of tablet in a patient's stomach.

- Talwar et al[46] developed a once-daily formulation for oral administration of ciprofloxacin. The formulation was composed of 69.9% ciprofloxacin base, 0.34% sodium alginate, 1.03% xanthum gum, 13.7% sodium bicarbonate, and 12.1% cross-linked poly vinyl pyrrolidine. The viscolysing agent initially and the gel-forming polymer later formed a hydrated gel matrix that entrapped the gas, causing the tablet to float and be retained in the stomach or upper part of the small intestine (spatial control). The hydrated gel matrix created a tortuous diffusion path for the drug, resulting in sustained release of the drug (temporal delivery).
- Two patents granted to Alza Corporation revealed a device having a hollow deformable unit that was convertible from a collapsed to expandable form and vice versa. The deformable unit was supported by a housing that was internally divided into 2 chambers separated by a pressure-sensitive movable bladder. The first chamber contained the therapeutic agent and the second contained a volatile liquid (cyclopentane, ether) that vaporized at body temperature and imparted buoyancy to the system. The system contained a bioerodible plug to aid in exit of the unit from the body.[47, 48]
- Baumgartner et al[49] developed a matrix-floating tablet incorporating a high dose of freely soluble drug. The formulation containing 54.7% of drug, HPMC K4 M, Avicel PH 101, and a gas-generating agent gave the best results. It took 30 seconds to become buoyant. In vivo experiments with fasted state beagle dogs revealed prolonged gastric residence time. On radiographic images made after 30 minutes of administration, the tablet was observed in animal's stomach and the next image taken at 1 hour showed that the tablet had altered its position and turned around. This was the evidence that the tablet did not adhere to the gastric mucosa. The MMC (phase during which large nondisintegrating particles or dosage forms are emptied from stomach to small intestine) of the gastric emptying cycle occurs approximately every 2 hours in humans and every 1 hour in dogs but the results showed that the mean gastric residence time of the tablets was 240 ± 60 minutes (n = 4) in dogs. The comparison of gastric motility and stomach emptying between humans and dogs showed no big difference and therefore it was speculated that the experimentally proven increased gastric residence time in

beagle dogs could be compared with known literature for humans, where this time is less than 2 hours.

- Moursy et al[50] developed sustained release floating capsules of nicardipine HCl. For floating, hydrocolloids of high viscosity grades were used and to aid in buoyancy sodium bicarbonate was added to allow evolution of CO_2. In vitro analysis of a commercially available 20-mg capsule of nicardipine HCl (MICARD) was performed for comparison. Results showed an increase in floating with increase in proportion of hydrocolloid. Inclusion of sodium bicarbonate increased buoyancy. The optimized sustained release floating capsule formulation was evaluated in vivo and compared with MICARD capsules using rabbits at a dose equivalent to a human dose of 40 mg. Drug duration after the administration of sustained release capsules significantly exceeded that of the MICARD capsules. In the latter case the drug was traced for 8 hours compared with 16 hours in former case.
- Atyabi and coworkers[51] developed a floating system using ion exchange resin that was loaded with bicarbonate by mixing the beads with 1 M sodium bicarbonate solution. The loaded beads were then surrounded by a semipermeable membrane to avoid sudden loss of CO_2. Upon coming in contact with gastric contents an exchange of chloride and bicarbonate ions took place that resulted in CO_2 generation thereby carrying beads toward the top of gastric contents and producing a floating layer of resin beads (Figure 4) .The in vivo behavior of the coated and uncoated beads was monitored using a single channel analyzing study in 12 healthy human volunteers by gamma radio scintigraphy. Studies showed that the gastric residence time was prolonged considerably (24 hours) compared with uncoated beads (1 to 3 hours).

  Non-effervescent floating dosage forms use a gel forming or swellable cellulose type of hydrocolloids, polysaccharides, and matrix-forming polymers like polycarbonate, polyacrylate, polymethacrylate, and polystyrene. The formulation method includes a simple approach of thoroughly mixing the drug and the gel-forming hydrocolloid. After oral administration this dosage form swells in contact with gastric fluids and attains a bulk density of < 1. The air entrapped within the

swollen matrix imparts buoyancy to the dosage form. The so formed swollen gel-like structure acts as a reservoir and allows sustained release of drug through the gelatinous mass.

- Thanoo et al[52] developed polycarbonate microspheres by solvent evaporation technique. Polycarbonate in dichloromethane was found to give hollow microspheres that floated on water and simulated biofluids as evidenced by scanning electron microscopy (SEM). High drug loading was achieved and drug-loaded microspheres were able to float on gastric and intestinal fluids. It was found that increasing the drug-to-polymer ratio increased both their mean particle size and release rate of drug.
- Nur and Zhang[53] developed floating tablets of captopril using HPMC (4000 and 15 000 cps) and carbopol 934P. In vitro buoyancy studies revealed that tablets of 2 kg/cm^2 hardness after immersion into the floating media floated immediately and tablets with hardness 4 kg/cm^2 sank for 3 to 4 minutes and then came to the surface. Tablets in both cases remained floating for 24 hours. The tablet with 8 kg/cm^2 hardness showed no floating capability. It was concluded that the buoyancy of the tablet is governed by both the swelling of the hydrocolloid particles on the tablet surface when it contacts the gastric fluids and the presence of internal voids in the center of the tablet (porosity). A prolonged release from these floating tablets was observed as compared with the conventional tablets and a 24-hour controlled release from the dosage form of captopril was achieved.
- Bulgarelli et al[54] studied the effect of matrix composition and process conditions on casein gelatin beads prepared by emulsification extraction method. Casein by virtue of its emulsifying properties causes incorporation of air bubbles and formation of large holes in the beads that act as air reservoirs in floating systems and serve as a simple and inexpensive material used in controlled oral drug delivery systems. It was observed that the percentage of casein in matrix increases the drug loading of both low and high porous matrices, although the loading efficiency of high porous matrices is lower than that of low porous matrices.
- Fell et al[55] prepared floating alginate beads incorporating amoxycillin. The beads were produced by dropwise addition of alginate into calcium chloride solution,

followed by removal of gel beads and freeze-drying. The beads containing the dissolved drug remained buoyant for 20 hours and high drug-loading levels were achieved.

- Streubel et al[56] prepared single-unit floating tablets based on polypropylene foam powder and matrix-forming polymer. Incorporation of highly porous foam powder in matrix tablets provided density much lower than the density of the release medium. A 17% wt/wt foam powder (based on mass of tablet) was achieved in vitro for at least 8 hours. It was concluded that varying the ratios of matrix-forming polymers and the foam powder could alter the drug release patterns effectively.
- Asmussen et al[57] invented a device for the controlled release of active compounds in the gastrointestinal tract with delayed pyloric passage, which expanded in contact with gastric fluids and the active agent was released from a multiparticulate preparation. It was claimed that the release of the active compound was better controlled when compared with conventional dosage forms with delayed pyloric passage.
- El-Kamel et al[58] prepared floating microparticles of ketoprofen, by emulsion solvent diffusion technique. Four different ratios of Eudragit S 100 with Eudragit RL were used. The formulation containing 1:1 ratio of the 2 above-mentioned polymers exhibited high percentage of floating particles in all the examined media as evidenced by the percentage of particles floated at different time intervals. This can be attributed to the low bulk density, high packing velocity, and high packing factor.
- Illum and Ping[59] developed microspheres that released the active agent in the stomach environment over a prolonged period of time. The active agent was encased in the inner core of microspheres along with the rate-controlling membrane of a water-insoluble polymer. The outer layer was composed of bioadhesive (chitosan). The microspheres were prepared by spray drying an oil/water or water/oil emulsion of the active agent, the water-insoluble polymer, and the cationic polymer.

- Streubel et al[60] developed floating microparticles composed of polypropylene foam, Eudragit S, ethyl cellulose (EC), and polymethyl metha acrylate (PMMA) and were prepared by solvent evaporation technique. High encapsulation efficiencies were observed and were independent of the theoretical drug loading. Good floating behavior was observed as more than 83% of microparticles were floating for at least 8 hours. The in vitro drug release was dependent upon the type of polymer used. At similar drug loading the release rates increased in the following order PMMA < EC < Eudragit S. This could be attributed to the different permeabilities of the drug in these polymers and the drug distribution within the system.
- Sheth and Tossounian[61] developed an HBS system containing a homogeneous mixture of drug and the hydrocolloid in a capsule, which upon contact with gastric fluid acquired and maintained a bulk density of less than 1 thereby being buoyant on the gastric contents of stomach until all the drug was released.
- Sheth and Tossounian[62] developed hydrodynamically balanced sustained release tablets containing drug and hydrophilic hydrocolloids, which on contact with gastric fluids at body temperature formed a soft gelatinous mass on the surface of the tablet and provided a water-impermeable colloid gel barrier on the surface of the tablets. The drug slowly released from the surface of the gelatinous mass that remained buoyant on gastric fluids
- Ushomaru et al[63] developed sustained release composition for a capsule containing mixture of cellulose derivative or a starch derivative that formed a gel in water and higher fatty acid glyceride and/or higher alcohol, which was solid at room temperature. The capsules were filled with the above mixture and heated to a temperature above the melting point of the fat components and then cooled and solidified.
- Bolton and Desai[64] developed a noncompressed sustained release tablet that remained afloat on gastric fluids. The tablet formulation comprised 75% of drug and 2% to 6.5% of gelling agent and water. The noncompressed tablet had a density of less than 1 and sufficient mechanical stability for production and handling.

- Kawashima et al[65] prepared multiple-unit hollow microspheres by emulsion solvent diffusion technique. Drug and acrylic polymer were dissolved in an ethanol-dichloromethane mixture, and poured into an aqueous solution of PVA with stirring to form emulsion droplets. The rate of drug release in micro balloons was controlled by changing the polymer-to-drug ratio. Microballoons were floatable in vitro for 12 hours when immersed in aqueous media. Radiographical studies proved that microballoons orally administered to humans were dispersed in the upper part of stomach and retained there for 3 hours against peristaltic movements.
- Dennis et al[66] invented a buoyant controlled release pharmaceutical powder formulation filled into capsules. It released a drug of a basic character at a controlled rate regardless of the pH of the environment. PH-dependent polymer is a salt of a polyuronic acid such as alginic acid and a pH-independent hydrocarbon gelling agent, hydroxypropylmethyl cellulose.
- Spickett et al[67] invented an antacid preparation having a prolonged gastric residence time. It comprised 2 phases. The internal phase consisted of a solid antacid and the external phase consisted of hydrophobic organic compounds (mono-, di-, and triglycerides) for floating and a non-ionic emulsifier.
- Franz and Oth[68] described a sustained release dosage form adapted to release of the drug over an extended period of time. It comprised a bilayer formulation in which one layer consisted of drug misoprostal and the other had a floating layer. The uncompressed bilayer formulation was kept in a capsule and was shown to be buoyant in the stomach for 13 hours. The dosage form was designed in such a way that the entire drug was released in the stomach itself.
- Wu et al[69] developed floating sustained release tablets of nimodipine by using HPMC and PEG 6000. Prior to formulation of floating tablets, nimodipine was incorporated into poloxamer-188 solid dispersion after which it was directly compressed into floating tablets. It was observed that by increasing the HPMC and decreasing the PEG 6000 content a decline in *invitro* release of nimodipine occurred.

- Wong et al[70] developed a prolonged release dosage form adapted for gastric retention using swellable polymers. It consisted of a band of insoluble material that prevented the covered portion of the polymer matrix from swelling and provided a segment of a dosage form that was of sufficient rigidity to withstand the contractions of the stomach and delayed the expulsion of the dosage form from the stomach.
- Mitra[71] developed a sustained release multilayered sheet-like medicament device. It was buoyant on the gastric contents and consisted of at least 1 dry, self-supporting carrier film of water-insoluble polymer. The drug was dispersed or dissolved in this layer and a barrier film overlaid the carrier film. The barrier film was compsosed of 1 water-insoluble layer and another water-soluble and drug-permeable polymer or copolymer layer. The 2 layers were sealed together in such a way that plurality of small air pockets was entrapped that gave buoyancy to the formulation.
- Harrigan[72] developed an intragastric floating drug delivery system that was composed of a drug reservoir encapsulated in a microporous compartment having pores on top and bottom surfaces. However, the peripheral walls were sealed to prevent any physical contact of the drug in the reservoir with the stomach walls.
- Joseph et al[73] developed a floating dosage form of piroxicam based on hollow polycarbonate microspheres. The microspheres were prepared by the solvent evaporation technique. Encapsulation efficiency of ~95% was achieved. In vivo studies were performed in healthy male albino rabbits. Pharmacokinetic analysis was derived from plasma concentration vs time plot and revealed that the bioavailability from the piroxicam microspheres alone was 1.4 times that of the free drug and 4.8 times that of a dosage form consisting of microspheres plus the loading dose and was capable of sustained delivery of the drug over a prolonged period.
- Kawashima et al[74] estimated the hollow structure of microspheres made of acrylic resins by measuring particle density (P_p) by a photographic counting method and a liquid displacement method. An image analyzer was used to determine the volume (v) of particles (n) of weight (w):

- Porosity was measured by $\epsilon = (1 - P_p / P_t) \times 100$, where P_t is the true density.
- Bulgarelli et al[75] developed casein gelatin beads and determined their porosity by mercury intrusion technique. The principle of this technique is that pressure (P) required to drive mercury through a pore decreases as described by the Washburn equation: $P = (-4 \sigma \cos \theta) d$, where d is the pore diameter, σ is mercury / air interfacial tension, and θ is the contact angle at mercury air pore wall interface.
- Sakuma et al[76] prepared radiolabeled anionic poly metha acrylic acid nanoparticles and the particle size of nonlabeleled nanoparticles was measured by dynamic spectrophotometry.
- *In vivo* gastric residence time of a floating dosage form is determined by X-ray diffraction studies, gamma scintigraphy,[77] or roentgenography
- Miyazaki et al[78] conducted pharmacokinetic studies on floating granules of indomethacin prepared with chitosan and compared the peak plasma concentration and AUC with the conventional commercially available capsules. It was concluded that the floating granules prepared with chitosan were superior in terms of decrease in peak plasma concentration and maintenance of drug in plasma.
- Ichikawa et al[79] developed a multiparticulate system that consisted of floating pills of a drug (p- amino benzoic acid) having a limited absorption site in the gastrointestinal tract. It was found to have 1.61 times greater AUC than the control pills
- Katayama et al[80] developed a sustained release (SR) liquid preparation of ampicillin containing sodium alginate, which spreads out and aids in adhering to the gastric mucosal surface. Thus, the drug is continuously released in the gastric region.
- Esharghi et al[81] developed a swellable asymmetric triple-layer tablet with floating ability to prolong the gastric residence time of triple drug regimen (tetracycline, metronidazole, clarithromycin) of Helicobacter pylori–associated peptic ulcers using HPMC and PEO as the rate-controlling polymeric membrane excipients. Results demonstrated that sustained delivery of tetracycline and metronidazole over 6 to 8 hours could be achieved while the tablets remained floating. It was

concluded that the developed delivery system had the potential to increase the efficacy of the therapy and improve patient compliance.

- Sato and Kawashima[82] developed microballoons of riboflavin, which could float in JP XIII no 1 solution (simulated gastric fluid). These were prepared by an emulsion solvent technique. To assess the usefulness of the intragastric floating property of the developed microballoons of riboflavin, riboflavin powder, nonfloating microspheres of riboflavin, and floating microballoons of riboflavin were administered to 3 volunteers. Riboflavin pharmacokinetics was assessed by urinary excretion data. It could be concluded that although excretion of riboflavin following administration of floating microballoons was not sustained in fasted state, it was significantly sustained in comparison to riboflavin powder and nonfloating microspheres in the fed state. This could be due to the reason that the nonfloating formulation passes through the proximal small intestine at once from where riboflavin is mostly absorbed, while the floating microballoons gradually sank in the stomach and then arrived in the proximal small intestine in a sustained manner. Total urinary excretion (%) of riboflavin from the floating microballoons was lower than that of riboflavin powder. This was attributed to incomplete release of riboflavin from microballoons at the site of absorption.
- Shimpi et al[83]studied the application of hydrophobic lipid, Gelucire 43/01 for the design of multi-unit floating systems of a highly water-soluble drug, diltiazem HCl. Diltiazem HCl-Gelucire 43/01 granules were prepared by the melt granulation technique. The granules were evaluated for in vitro and in vivo floating ability, surface topography, and *in vitro* drug release. *In vivo* floating ability was studied by γ-scintigraphy in 6 healthy human volunteers and the results showed that the formulation remained in the stomach for 6 hours. It could be concluded that Gelucire 43/01 can be considered as an effective carrier for design of a multi-unit FDDS of highly water-soluble drugs such as diltiazem HCl.
- A gastroretentive drug delivery system of ranitidine hydrochloride was designed using guar gum, xanthan gum, and hydroxy propyl methyl cellulose. Sodium bicarbonate was incorporated as a gas-generating agent[84] . The effect of citric acid and stearic acid on drug release profile and floating properties was

investigated. The addition of stearic acid reduces the drug dissolution due to its hydrophobic nature. A 3^2 full factorial design was applied to systemically optimize the drug release profile and the results showed that a low amount of citric acid and a high amount of stearic acid favor sustained release of ranitidine hydrochloride from a gastroretentive formulation. Hence, it could be concluded that a proper balance between a release rate enhancer and a release rate retardant could produce a drug dissolution profile similar to a theoretical dissolution profile of ranitidine hydrochloride.

- In a recent work by Sriamornsak et al,[85] a new emulsion-gelation method was used to prepare oil-entrapped calcium pectinate gel (CaPG) beads as a carrier for intragastric floating drug delivery. The gel beads containing edible oil were prepared by gently mixing or homogenizing an oil phase and water phase containing pectin, and then extruded into calcium chloride solution with gentle agitation at room temperature. The oil-entrapped calcium pectinate gel beads floated if a sufficient amount of oil was used. Scanning electron photomicrographs demonstrated very small pores, ranging between 5 and 40 μm, dispersed all over the beads. The type and percentage of oil played an important role in controlling the floating of oil-entrapped CaPG beads. The oil-entrapped CaPG beads were a good choice as a carrier for intragastric floating drug delivery.
- Reddy and Murthy[86] have discussed advantages and various disadvantages of single- and multiple-unit hydrodynamic systems
- Many gastro retentive dosage forms are designed to overcome the natural physiology and remain in the fasted stomach[87 - 95] during the migrating motor complex (MMC). The mechanisms of retention often rely on rapid expansion by either gas generation, mechanical means or swelling to at least the size of a golf ball, approximately 25 to 30 mm diameter, followed by a collapse or degradation to a reduced size at some durationafter the drug is delivered. These approaches have been somewhat successful. However, additional studies of a larger population are required, especially in light of some variable emptying in smaller studies.

- In the fed state[96] the closing and contracting of the pylorus with a mean diameter of approximately 1.2 cm and regular grinding waves of much smaller amplitude than in the fasted state are a mechanism to digest food by retaining large particles in the stomach until reduced in size.
- In the fasted state [97] approximately every 90 minutes a full amplitude series of waves, phase III of the MMC cycle or house keeper wave empties the total contents of the stomach. Food, particularly fatty acids, interrupts the recurrence of this housekeeper wave and prevents emptying of the stomach.
- Meyer et al [98] found that spheres smaller than a diameter of 1.6 mm emptied either earlier or at the same time as the nutrient part of the meal. However, spheres with a diameter equal to or spheres with a diameter equal to or greater than 2.4 mm emptied slower than the liver meal. Interestingly, at a sphere diameter of 5 mm, very little emptying took place up to about 180 minutes postprandial.
- Diao *et al*[99] designed a miokamycin floating sustained –action tablets based on hydro dynamically balanced drug delivery system. It reveals that relative bioavailability was greatly increased over the syrup.
- Desai *et al*[100] compared a floating controlled release theophylline tablets with conventional theophylline tablets. The *in vivo* bioavailability studies showed that floating tablets maintain constant theophylline release of 2 mg/ml for 24 h.
- Du *et al*[101] prepared a floating cinnarizine capsules based on hydrodynamic balanced system. Their floating capability, drug release profile and stability were tested. In the artificial gastric juice, capsules floated up to 8 h and the drug was released slowly.
- Elkheshen *et al*[102] prepared a sustained release floating system for verapamil hydrochloride using different polymers, which are HPMC, HPC and ethyl cellulose. Floating was maintained by adding effervescent mixture of sodium bicarbonate and citric acid. Results showed that HPMC has good floating property.
- Gabr *et al*[103] prepared buffered buoyant furosemide capsule formulation. Incorporation of HPMC in the formulation showed a good sustained drug release

and duration of buoyancy. Drug bioavailability was significantly increased for buffered floating capsule when compared with commercially tablet.

- Gibaly EI *et al*[104] prepared floating microspheres containing melatonin by ionic interaction of chitosan and negatively charged surfactant sodium di octyl sulfosuccinate. The characteristics of the floating microspheres were compared with conventional non-floating microsphere, which was manufactured by using chitosan, and sodium tripolyphosphate was investigated. The dissolution profiles showed that floating microspheres release the drug for several hours.
- Hilton *et al*[105] developed a sustain release dosage form of amoxycillin trihydrate based on single matrix tablet. The tablets remained buoyant for 6 h and had sustained drug release.
- Jain SK *et al*[106] showed gastro retentive dosage forms had the potential to be used as controlled release system. A controlled release system designed to increase its residence time in the stomach without contact with the mucosa was achieved through the preparation of floating microspheres by the emulsion solvent diffusion technique consisting of calcium silicate as porous carrier, repaglinide as drug and Eudradgit as polymer. The designed system combines excellent buoyant ability and good sustained drug release pattern.
- Kandam SS *et al*[107] designed gastro retentive floating capsule using hydrophilic polymer with dilitiazem HCl. HPMC K-4M and HPMC-15m, and lactose was added. Capsules were evaluated for lag time, floating time, and *Invitro* drug release.
- Lin N *et al et al*[108] developed aspirin sustained release floating capsule. *In vitro* dissolution and *in vivo* studies showed good sustained release of the drug. There was also decreased G.I. Irritation and side effects of the drug
- Brener *et al*[109] reviewed about the gastroretentive drug delivery systems and the various factor affecting the drug delivery systems.
- Murata *et al*[110] prepared two types of alginate gel beads capable of floating in stomach. Metronidazole was used as model drug. The *in vivo* release in guinea pig showed that concentration of drug in gastric mucosa was higher with floating alginate gel beads when compared with solution.

- Narendra *et al*[111] developed an optimized gastric floating dosage form containing metoprolol tartate as model drug. The results showed that total polymer content to drug ratio and polymer to polymer ratio significantly affect the floating time and drug release properties of formulated bilayer tablets.
- Regeni *et al*[112] prepared moricizine HCl sustained release tablet which float and release the drug for 6 h. Plasma concentration time curve showed that tablet exhibit typical sustained release profile.
- Sahni *et al*[113] prepared hydrodynamic balanced system of paracetamol using HPMC-K4m as hydrophilic polymer. The results showed that tablet float and release the drug for 12 hours with liquid paraffin as the best release modifier.
- Klausner *et al*[114] studied the pharmacokinetics and pharmacodynamics behavior of furosemide after giving the gastroretentive system of the drug to healthy human volunteer
- Talukder *et al*[115] developed a floatable multi particulate system with potential for intragastric sustained drug delivery. Cross-linked beads were made by using low methoxylated pectin and sodium alginate. Riboflavin, tetracycline and Methotrexate were used as models drugs for encapsulation. It appeared that the nature of cross-linking, drug solubility and production approach were important and provide the opportunity and potential for development of a gastro retentive drug delivery system.
- Tu *et al*[116] prepared oral sustained released floating tablets of norfloxacin. Gamma scintigraphy showed that floating tablets retained in the human stomach was longer (5 - 6 h) when compared with commercial capsules (0.5 - 1 h).
- Viral Patel *et al*[117] developed an intragastric dosage form of cefuroxime axetil. HPMC K-4M, HPMC K-100LV and sodium lauryl sulfate were used to formulate the tablet by direct compression technology. The tablets were evaluated for *in vitro* buoyancy and drug release. It was cleared that for the development of controlled release form of poorly soluble drugs required a different viscosity grade of HPMC polymers and surfactant to impart the hydrophilic environment and wettability to drug molecules. Tablets showed good drug release profile.

- Whitehead *et al*[118] studied GI transit by using radio labeled freeze dried calcium alginate. Radio labeled freeze-dried calcium alginate multiple unit floating bead formulation was compared with radio labeled calcium alginate multiple unit non-floating bead formulation. The floating beads showed prolonged G.I transit time of 5.5 h.
- Ozdemis *et al*[119] prepared the floating dosage to enhance the bioavailability of furosemide. The solubility of furosemide was increased by forming inclusion complex with betadex (beta cyclodexterin). Dissolution studies and floating time were studied. The plasma AUC values of floating dosage forms were about 1.8 times greater than those of the conventional tablet
- Beta cyclo dextrin (β-CD) enhanced the release rate of poorly soluble naproxen and ketoprofen [120] from inert acrylic resins and hydrophilic swellable (high-viscosity hydroxy propyl methyl cellulose [HPMC]) tableted matrices.
- β-CD also enhanced the release of theophylline from HPMC matrix by increasing the apparent solubility and dissolution rate of the drug.[121]
- CDs increased the bioavailability of lipophilic itraconazole from both an oral solution and an intravenous formulation by improving the drug solubility and absorption.[122]
- Reduction of drug crystallinity on complexation or solid dispersion with CDs also contributes to the CD increased apparent drug solubility and dissolution rate.[123,124]
- CDs, as a result of their ability to form in situ inclusion complexes in dissolution medium, can enhance drug dissolution even when there is no complexation in the solid state.[125]
- CDs have been used to ameliorate the irritation caused by drugs.[126]
- The increased drug efficacy and potency (ie, reduction of the dose required for optimum therapeutic activity), caused by CD-increased drug solubility, may reduce drug toxicity by making the drug effective at lower doses. β-CD enhanced the antiviral activity of ganciclovir on human cytomegalovirus clinical strains and the resultant increase in the drug potency reduced the drug toxicity.[127]
- The toxicities associated with crystallization of poorly water-soluble drugs in parenteral formulations can often be reduced by formation of soluble drug:CD

complexes. Formulation of phenytoin with HP2-β-CD showed considerably reduced tissue irritation compared with a commercial injection of the drug in a BALB/c mouse model.[128]

- CD entrapment of drugs at the molecular level prevents their direct contact with biological membranes and thus reduces their side effects (by decreasing drug entry into the cells of nontargeted tissues) and local irritation with no drastic loss of therapeutic benefits.[129]
- CDs were reported to enhance the physical stability of viral vectors for gene therapy, and the formulations containing sucrose and CDs were stable for 2 years when stored at 20°C.[130]
- Complexation can also mask the undesirable taste of drugs. Complexation with CDs suppressed the bitter taste of oxyphenonium bromide. With the assumption that only the free drug molecule exhibits bitter taste, the extent of the suppression was reported to be dependent on the availability of free drug, regardless of the kind and concentration of CD.[131]
- Quaglia et al[132] reported that CDs can be used to modulate drug delivery from swellable systems, eg, β-CD significantly affected the delivery of nicardipine from swellable crosslinked polyethylene glycol matrix by decreasing effective drug diffusivity through the matrix.
- β-CD improved insulin loading of alginate microspheres prepared by an emulsion-based process. The process was suggested to be useful in the development of an oral insulin drug delivery system as the absorption of insulin from optimized microspheres was found to take place from the GI region. [133,134]
- Ferguson et al [135] states that 5 fluorouracil an inhibitor of thymidylate synthase, was shown to be more cytotoxic when combined with antisense oligodeoxy nucleotides specific for thymidylate synthase m RNA.
- Wang et al [136] formulated 5 fu with a synthetic nucleotide sequence. It showed a significantly enhanced effect on hepatoma cell growth as compared to 5 fu alone.
- Parker and cheng[137] infers that cytotoxic drugs like 5 fu are potent but they tend to exhibit side effects in the body.

- Ardalan and Glazer[138] states that in the case of 5 fu, it is rapidly absorbed through the blood capillaries into systemic circulation. This results in relatively low levels of drug near the site of action with the subsequent loss of efficacy and increased risk of systemic toxicity
- Brem, Lawson and Hagiwara et al[139] infers that by using sustained release formulations of 5 fu the incidence of side effects may be reduced and therapeutics effects increased.
- Shishu et al[140] formulated a multiple unit type oral floating dosage form (FDF) of 5 fluorouracil to prolong gastric residence time, target stomach cancer and increase bioavailability of drug. The multiple bead FDF was found to reduce the tumour incidence in mice by 74 %, while the conventional tablet dosage form reduced this incidence by only 25%.
- Truter EJ.[141] study the release behaviour of heat-stabilized albumin microspheres with entrapped 5-Fluorouracil (5-FU) in vitro, and determine the organ distribution in vivo, for potential application in the treatment of ovarian cancer. Additionally, blood chemistry and haematological profiles were composed after intraperitoneal administration of 5-FU-loaded albumin microspheres into adult female Wistar rats. The data suggest that 5-FU-loaded albumin microspheres may be beneficial in reducing the severe side-effects of this antimetabolite, whilst still maintaining therapeutic levels to cause tumour cell death.
- Cui-Yun et al[142] studied Alginate based microparticle drug delivery systems were prepared for the sustained release of antineoplastic drugs. Two drugs, 5-fluorouracil (5-FU) and tegafur, were encapsulated into the microparticles. The effect of the reinforcement conditions on the drug release property of the microparticles was studied, and the optimized concentration of chitosan solution for reinforcement was identified. The effects of drug feeding concentration and pH value of the release medium on the drug release were investigated.
- Molecular imprinting is a new and rapidly evolving technique[143] used to create synthetic receptors and it possesses great potential in a number of applications in the life sciences. Keeping in mind the therapeutic importance of 5-fluorouracil (5-FU) and the technological significance of molecular imprinting polymers, the

present study is an attempt to synthesize 2-hydroxyethylmetacrylate- and acrylic acid-based 5-FU imprinted hydrogels. Both molecular imprinted polymers (MIPs) and non-imprinted polymers were synthesized at the optimum crosslinker concentration obtained from swelling studies and used to study their recognition affinity, their swelling and the in vitro release dynamics of the drug. It was observed from this study that the recognition affinity of MIPs is increased when these are synthesized in a high concentration template solution.

- The authors[144] have developed a new method of drug delivery into the brain using implantable biodegradable microspheres. In this study, this method was used to provide localized and sustained delivery of 5-fluorouracil (5-FU) after the surgical resection of glioblastoma. This study demonstrates that biodegradable microspheres are efficient systems for drug delivery into the brain and may have future application in the treatment ofbraintumors.
- The use of biodegradable nanoparticles loaded with 5-fluorouracil[145] was investigated as a potential means to sustain the release of this drug. Nanoparticles prepared from four biodegradable polymers were loaded with 5-fluorouracil using three loading concentrations of drug and three different concentrations of added polymer. Drug release from nanoparticles was evaluated using a Franz cell diffusion apparatus, which showed an initial burst effect followed by a slower release phase over 24 h. Indeed, nanoparticles prepared from poly(lactide-co-glycolide) released 66% of their 5-fluorouracil payload over this period.
- Nakamura J, et al[146] performed the study to elucidate the stomach- and site-selective delivery of 5-fluorouracil (5-FU) following its application on the gastric serosal surface in rats. An experimental system utilizing a cylindrical diffusion cell attached to the gastric serosal surface was established. To evaluate the gastric distribution of 5-FU, the stomach was separated into the site under the diffusion cell (site 1) and the site not under the diffusion cell (site 2). Furthermore, the mucosal side at site 1 was separated from the serosal side. After intravenous and oral administration of 5-FU, the 5-FU concentrations at sites 1 and 2 until 240 min were similar. After gastric serosal surface application of 5-FU, however, the concentration of 5-FU at site 1 until 240 min was approximately 10-fold higher

than that at site 2, and was sustained. Furthermore, the 5-FU concentration on the mucosal side at site 1 and the serosal side at site 1 were comparable after gastric serosal surface application. The blood concentration of 5-FU was low (<4.4 microg/ml) until 240 min after gastric serosal surface application. The maximum blood concentration of 5-FU after gastric serosal surface application was significantly lower than after intravenous administration. Thus, the stomach- and site-selective delivery system following application on the gastric serosal surface could be applied with anticancer drugs for the treatment of gastric cancer.

- Sugitachi A et al[147] devised a muco-adhesive anticancer drug delivery system using 70% deacetylated chitin (DAC-70) and cisplatin (CDDP) and 5-fluorouracil (5-FU). The adhesive force between the system and human colonic mucosa was measured ex vivo, and a release profile of each drug was examined in vitro. Each system demonstrated a stronger muco-adhesive force at 37 degrees C than that of 25 degrees C. The CDDP-loaded system showed a sustained release of the drug while the 5-FU-loaded system exhibited an initial bursting of the agent. We presume that the release profile of CDDP and 5-FU is closely related to both degradability of the chitin and interactions between the chitin and each drug. The DAC-70/CDDP system would be clinically promising in loco-regional cancer chemotherapy.
- This study covers the preparation of the gelatin microsphere (GM)-anti-bovine serum albumin (anti-BSA) conjugate[148] for the development of a drug targeting approach for anticancer drug delivery. Microspheres of 5% (w/v) gelatin content were prepared by crosslinking with glutaraldehyde (GTA) at 0.05 and 0.50% (v/v) concentration. Microspheres were in the size range of 71-141?microm. The suitability of these microspheres as drug carriers for anticancer drug delivery was investigated in vitro by studying the release profiles of loaded methotrexate (MTX) and 5-fluorouracil (5-FU) and the cytotoxicities on cancer cell lines. Results indicated approximately 80% binding with conjugated anti-BSA and BSA-FITC. Based on their low cytotoxicity and the high antigen binding efficiencies, anti-BSA conjugated gelatin microspheres could be suitable targeted

drug carrier systems for selective and long-term delivery of anticancer drugs to a specific body compartment.

- Combination therapy of Antisense oligonucleotides (AODNs) and cytotoxic agents using biodegradable polymeric delivery systems[149] potentially offers several advantages including site-specific or organ-directed targeting, protection from digesting enzymes, and improved pharmacokinetics/pharmacodynamics resulting from sustained delivery of the entrapped drugs. Using a model AODN targeting the epidermal growth factor receptor (that is over-expressed in several cancers including breast and brain cancer) and the commonly used cytotoxic agent, 5-fluorouracil (5-FU), the authors have examined the use of poly (lactide-co-glycolide) (P(LA-GA)) microsphere formulations for co-delivery of these agents.Data suggest that by mixing individual formulations of 5-FU and AODNs at different mass ratios allowed greater flexibility in achieving the desired release profile as well as avoiding potential drug–drug interactions.

OBJECTIVE AND SCOPE OF RESEARCH WORK

In recent years considerable attention has been focused on the development of controlled release drug delivery systems. Controlled release drug delivery systems (CRDDS) are designed to release one or more drugs continuously in a predetermined pattern for a fixed period of time either systemically or to a specific target organ. The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Ehrlich, who proposed drug delivery to be as a “magic bullet”. It was the very first report published in 1902, on targeting, describing targeted drug delivery as an event where, a drug- carrier complex/conjugate, delivers drug(s) exclusively to the preselected target cells in a specific manner. Bangham’s observation on phospholipids hexagonal liquid crystals, that they are perm selective to the ions in a manner similar to biomembrane, led to discovery of artificial vesicular system based on phospholipid amphiphiles. Targeted drug delivery (TDD) implies for selective and effective localization of pharmacologically active moiety at preidentified (preselected) target(s) in therapeutic concentration, while restricting its access to non-target normal cellular linings, thus minimizing toxic effects and maximizing therapeutic index

Rationale OF Drug Targeting

The site specific targeted drug delivery negotiates an exclusive delivery to specific pre identified compartments with maximum intrinsic activity of drugs and concomitantly reduced access of drug to irrelevant non target cells. The targeted delivery to previously in-accessible domains, e.g., intracellular sites, virus, bacteria and parasites offers distinctive therapeutic benefits. The controlled rate and mode of drug delivery to pharmacological receptor and specific binding with target cells; as well as bioenvironmental protection of the drug en route to the site of action are specific features of targeting. Invariably, every event stated contributes to higher drug concentration at the site of action and resultant lower concentration at non target tissue where toxicity might crop up. The high drug concentration at the target site is a result of the relative cellular uptake of the drug vehicle, liberation of drug and efflux of free drug from the target site

Carrier is one of the most important entities essentially required for successful transportation of the loaded drug. They are drug vectors, which sequester, transport and retain

drug en route, while elute or deliver it within of in the vicinity of target. Carriers can do so either through an inherent characteristics or acquired (through structural modification), to interact selectively with biological targets, or otherwise they are engineered to release the drug in the proximity of target cell lines demanding optimal pharmacological action (therapeutic index).

An ideal drug carrier engineered as a targetable device should have the following features:

- It must be able to cross anatomical barriers and in case of tumour chemotherapy, tumour vasculature.
- It must be recognized specifically and selectively by the target cells and must maintain the avidity and specificity of the surface ligands
- The linkage of the drug and the directing unit (ligand) should be stable in plasma, interstitial and other biofluids.
- Carrier should be non-toxic, non immunogenic and biodegradable particulate or macromolecule and after recognition and internalization, the carrier system should release the drug moiety inside the target organs, tissues or cells.

The primary objectives of targeted drug delivery are to ensure safety and to improve efficacy of drugs as well as patient compliance. This is achieved by better control of plasma drug levels and less frequent dosing. CRDDS have been designed for oral, parentral, implantation and transdermal routes.

Oral route is the most desirable and preferred method of administering therapeutic agent for their systematic effects such as patient acceptance, convenience in administration, and cost-effective manufacturing process. Thus, a wide variety of approaches of drug delivery system (DDS) have been investigated for oral application. However, the development process is precluded by several physiological difficulties, such as an inability to restrain and localize the DDS within desired regions of gastrointestinal tract and highly variable nature of gastric emptying process. For example, the relatively brief gastric emptying time (GET) can result in incomplete drug release from the DD devices leading to diminished efficacy of the administered dose.

Intragastric floating drug delivery system (FDDS) is noted as one of the orally applicable DDS for prolongation of the GET. The bulk density of FDDS is lower than that of gastric fluids and thus it remains buoyant on stomach contents for a long time in the drug releasing process. Hence, it is useful for obtaining the sufficient bioavailability and the effective “plasma” level. In addition, FDDS is one of the optimal systems for stomach mucosa targeting of antitumor agent for the treatment of stomach cancer and antibiotics for the eradication of Helicobacter pylori.

In the present investigation design of gastroretentive floating tablets and floating hollow microspheres of 5 fluorouracil for oral controlled released is aimed.

One of the major antimetabolites used in a variety of solid cancers, such as stomach, colon, lung, and breast cancer, is 5-fluorouracil (5-FU). It is usually given intravenously, as absorption of 5-FU from the gastrointestinal tract is erratic and unpredictable. The intravenous route of administration is associated with severe systemic, dose related side effects because of 5-FU’s cytotoxic nature, when it reaches unwanted sites. After oral administration, gastrointestinal absorption is rapid, and peak levels in the blood are reached between 15 and 60 minutes after ingestion, but much variability is seen between individuals. After intravenous administration, the drug diffuses equally in all the compartments in a volume equivalent to the body fluid volume. Peak plasma levels are reached within minutes, and plasma half-life is 10 to 20 minutes. The drug, despite low lipid solubility, enters the cerebrospinal fluid and the brain.

Therefore, a stomach-specific single unit and multiple-unit FDF of 5-FU has been designed to reduce the dose and to reduce its unwanted dose dependent side effects, at other sites, by targeted delivery to gastric tumors. This stomach-specific oral delivery or the targeted drug delivery of fluorouracil for the stomach cancer, may serve as an alternative to inconvenient and painful conventional intravenous therapy.

PLAN OF WORK

Keeping the above objectives in view the work was planned on the following lines.

1. PREFORMULATION STUDIES

Analysis of 5 fluoro uracil

- **By U.V Spectroscopy**
- **By I.R Spectroscopy**
- **Melting Point**
- **Loss on Drying**
- **Determination of Bulk and Tapped Density**
- **Compressibility Index**
- **Angle of Repose**
- **Residue on Ignition**

Drug Excipients compatibility study

Drug Excipients compatibility study by I.R Spectroscopy

Preparation of Standard Graph of 5 Fluoro Uracil

2. STUDIES ON COMPLEXATION OF FLUOROURACIL WITH CYCLODEXTRIN

Phase-Solubility Study

PREPARATION OF SOLID COMPLEXES

- **Physical Mixture**
- **Kneading Method**
- **Freeze-Drying Method**
- **Co Evaporation Method**

EVALUATION

- **Fourier Transform Infrared (FTIR) Spectroscopy**
- **Differential Scanning Calorimetry**
- **Dissolution Rate Studies**
- **Molecular-Modeling Studies**

3. FORMULATION AND EVALUATION OF FLOATING ORAL CONTROLLED RELEASE TABLETS OF FLUOROURACIL

OPTIMIZATION OF TABLET PROCESS PARAMETERS:

GENERAL PROCEDURE FOR PREPARATION OF 5 FLUORO URACIL GRS TABLETS (FI –FXII)

CHARACTERIZATION OF 5 FU GRS GRANULES

- **Determination of Bulk and Tapped Density**
- **Angle of Repose**
- **Compressibility Index**
- **Loss on Drying**

CHARACTERIZATION OF 5 FU GRS TABLET (FI – FXII)

- **Tablet Size**
- **Hardness test**
- **Friability test**
- **Weight variation test**
- **Buoyancy determination**
- **Drug content (assay)**
- ***In vitro* Dissolution of Fabricated Tablets (FI - FXII)**
- **Kinetic studies**

REFABRICATION AND EVALUATION OF SELECTED FORMULATIONS

- **Formulation of selected tablet to check the reproducibility**

- **Physical characteristics of the formulation**
- **Buoyancy determination**
- ***In vitro* dissolution profile of selected 5 FU GRS tablets**
- **Drug-excipients interaction study of selected formula by Infra red spectroscopy**
- **Kinetic Studies (Mathematical model)**

4. FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF FLUOROURACIL

IR Spectroscopy

Thermal analysis

PREPARATION OF MICROSPHERES

EVALUATION OF MICROSPHERES

- **Determination of percentage yield value of microspheres**
- **Drug loading and drug entrapment efficiency**
- **Particle size and size distribution**
- **Morphology**
- **Floating behavior**
- ***In vitro* release study of the microspheres**
- **Mathematical model**

5. *INVIVO* EVALUATION OF THE SELECTED FORMULATIONS OF TABLET AND MICROSPHERES

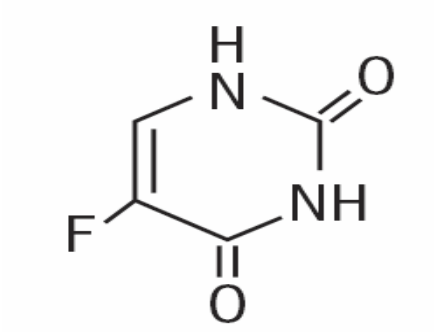
***INVIVO* FLOATING STUDY**

***IN VIVO* ANTITUMOUR ACTIVITY OF SELECTED FORMULATIONS ON EHRLICH ASCITES CARCINOMA IN MICE**

6. STABILITY STUDY OF THE SELECTED FORMULATION.

DRUG PROFILE

Fluorouracil



$C_4H_3FN_2O_2$ 130.08

2, 4 (1H, 3H)-Pyrimidinedione, 5-Fluoro-, 5-Fluorouracil

Fluorouracil contains not less than 98.5 percent and not more than 101.0 percent of $C_4H_3FN_2O_2$, calculated on the dried basis.

Caution-Great care should be taken to prevent inhaling particles of Fluorouracil and exposing the skin to it.

Packaging and Storage – Preserve in tight, light-resistant containers.

Reference Standard – USP Fluorouracil Reference Standard – Dry in vacuum over phosphorus pentoxide at 80° for 4 hours before using.

Identification

A: The infrared absorption spectrum of a mineral oil dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Fluorouracil RS[150].

B: The ultraviolet absorption spectrum of a 1 in 100,000 solution in a pH 4.7 acetate buffer (prepared from 8.4 g of sodium acetate and 3.35 ml of glacial acetic acid mixed with water to make 1000 ml) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Fluorouracil RS, concomitantly measured, and the respective absorptivities, calculated on the dried basis at the wavelength of maximum absorbance at about 266 nm, do not differ by more than 3.0%.

C: To 5 ml of a solution (1 in 100) add 1 ml of bromine water TS: was added the bromine color is discharged.

Loss on Drying - Dried in vacuum over phosphorus pentoxide at 80° for 4 hours: it loses not more than 0.5% of its weight.

Residue on Ignition : Not more than 0.1%.

Heavy Metals : 0.002%.

Acidity: pH of a 1% w/v solution, 4.5 to 5.0

Assay

0.1 N Tetrabutyl ammonium hydroxide in methanol - A commercially available solution of tetrabutylammonium hydroxide in methanol was diluted with methanol and standardized Tenth-Normal (0.1 N).

Procedure - About 400 mg of 5 Fluorouracil, accurately weighed, was transferred to a 250-ml conical flask, 80 ml of dimethylformamide was added and warmed gently to dissolve and cooled. 5 drops of a 1 in 100 solution of thymol blue in dimethylformamide was added, and titrated with 0.1 N Tetrabutylammonium hydroxide in methanol to a blue end-point, taking precautions to prevent absorption of atmospheric carbon dioxide. A blank determination was performed and any necessary correction was made. Each ml of 0.1 N tetrabutylammonium hydroxide is equivalent to 13.01 mg of $C_2H_3FN_2O_2$.

Mechanism of Action

5-FU requires enzymatic conversion to the nucleotide (ribosylation and phosphorylation) in order to exert its cytotoxic activity. Several routes are available for the formation of the 5'-monophosphate nucleotide (F-UMP) in animal cells. 5-FU may be converted to fluorouridine by uridine phosphorylase and then to F-UMP by uridine kinase, or it may react directly with 5-phosphoribosyl-1-pyrophosphate (PRPP), in a reaction catalyzed by the enzyme orotate phosphoribosyl transferase, to form F-UMP. Many metabolic pathways are available to F-UMP, including incorporation into RNA. A reaction sequence crucial for antineoplastic activity involves reduction of the diphosphate nucleotide by the enzyme ribonucleotide diphosphate reductase to the deoxynucleotide level and the eventual formation of 5-fluoro-2'-deoxyuridine-5'-phosphate (F-dUMP). F-FU also may be converted directly to the deoxyriboside 5-FUdR by the enzyme thymidine phosphorylase and further to F-dUMP, a potent inhibitor of thymidylate synthesis, by thymidine kinase.

Adverse Effects and Treatment

The toxic effects of fluorouracil may be severe and sometimes fatal. Leucopenia is the main dose-limiting effect[151] and the occurrence of stomatitis or severe diarrhoea is early signs that treatment should be stopped. Depression of the white-cell count is greatest after 7 to 20 days and counts may return to normal after about 30 days.

Thrombocytopenia is usually at a maximum of 7 to 17 days after the first dose. Reducing the rate of injection to a slow infusion over 2 to 8 hours can decrease the toxicity but this may be less effective than administration by rapid injection.Local inflammatory and photosensitivity reactions have occurred following topical use.

Absorption and Fate

Absorption of fluorouracil from the gastro-intestinal tract is unpredictable and fluorouracil is usually given intravenously. Little is absorbed when fluorouracil is applied to healthy skin but up to 20% of a dose applied to diseased skin may be excreted in the urine over 24 hours. It is also absorbed to a small extent through serous membranes.

After intravenous injection fluorouracil is cleared rapidly from plasma. It is distributed throughout body tissues and fluids including the cerebrospinal fluid and malignant effusions, and disappears from the plasma within about 3 hours. Fluorouracil is converted to active nucleotide metabolites within the target cell itself. About 15% of an intravenous dose is excreted unchanged in the urine within 6 hours. The remainder is inactivated primarily in the liver and is catabolised similarly to endogenous uracil. A large amount is excreted as respiratory carbon dioxide; urea is also produced.

Pharmacokinetic features of fluorouracil

The elimination half-life of fluorouracil from plasma is about 10 minutes and plasma concentrations cannot be detected 2 hours after an intravenous dose. Nevertheless, the activity of fluorouracil may persist for several days following a single intravenous injection. Studies in animals have shown that active metabolite 5-fluoro-2'deoxyuridine 5'-monophosphate (FdUMP) remains in tissues over several days and a terminal elimination half-life for fluorouracil of 20 hours has been reported in rats by C. Finn and W. Sadee. Erratic concentrations of fluorouracil have followed oral administration with peak plasma concentrations between 0.8 and 40 µg per ml 10 minutes to 2 hours after a dose of 15mg per kg body-weight. The active fluorouracil nucleotides are trapped within the cell because of their high polarity and direct measurements of metabolites in target and host tissues appear to be necessary to obtain a link between drug concentrations and response.

Peak plasma concentration ranged from 24 to 125 µg per ml with an elimination half-life of 10 to 30 minutes following the intravenous bolus injection of fluorouracil in 12 cancer patients. Doses ranged from 9 to 16 mg per kg body-weight. When the same dose was given by mouth to these patients plasma concentrations were below 10 µg per ml and bioavailability ranged from 0 to about 75% but was usually increased markedly if the dose was doubled. No correlation was found between liver abnormalities and bioavailability. Plasma concentrations ranged from 0 to 8 µg per ml in 6 patients given fluorouracil 20 to 30 mg per kg body-weight daily by slow intravenous infusion on 5

consecutive days. In 4 patients fluorouracil could not be detected in the plasma after rectal administration by enema in 100 ml of saline.

Uses. Fluorouracil, a pyrimidine analogue, is an antineoplastic agent which acts as an antimetabolite to uracil. After intracellular conversion to the active deoxynucleotide it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. It can also interfere with RNA synthesis. It also has immunosuppressant properties. Fluorouracil is used in the palliation of inoperable malignant neoplasms, especially those of the gastro-intestinal tract, breast, liver, and pancreas. It is often used with cyclophosphamide and methotrexate in the combination chemotherapy of breast cancer. Therapeutic doses are close to toxic levels and fatalities have occurred. Initial treatment should always be given in hospital.

A usual dose by intravenous injection is 12 mg per kg body-weight daily to a maximum of 1 g daily for 3 or 4 days. If there is no evidence of toxicity[152], this is followed after 1 day by 6 mg per kg on alternate days for 3 or 4 further doses. An alternative schedule is to give 15 mg per kg intravenously once a week throughout the course. Maintenance is usually with 5 to 15 mg per kg weekly. Fluorouracil may also be given by intravenous infusion, 15 mg per kg daily, to a maximum of 1 g daily, being infused in 500 ml of dextrose injection over 4 hours and repeated on successive days until toxicity occurs or a total of 12 to 15 g has been give. The course may be repeated after 4 to 6 weeks. It is also given by intra-arterial infusion and by mouth.

The white cell count should be determined daily during treatment with fluorouracil and therapy stopped immediately if the count falls rapidly or falls to below 3500 per mm^3, if the platelet count falls below 100 000 per mm^3, or if adverse effects occur.

Fluorouracil is used topically in the treatment of solar or actinic keratoses and other tumours of the skin including Bowen’s disease and superficial basal cell carcinomas, usually as a 5% cream or ointment or as a 1 to 5% solution in propylene glycol.

Preparations

Fluorouracil Cream (U.S.P.). A cream containing fluorouracil. It may contain sodium hydroxide to adjust the pH. Store at 15° to 30° in airtight containers.

Fluorouracil Injection (U.S.P). A sterile solution in Water for Injections, prepared with the aid of sodium hydroxide. It contains 45 to 55 mg of fluorouracil in each ml. pH 8.6 to 9. Store at 15° to 30°. Avoid freezing; protect from light. If a precipitate forms, redissolve by warming to 60°, with shaking, and cool.

Fluorouracil Topical Solution (U.S.P.). A solution of fluorouracil. It may contain sodium hydroxide to adjust the pH. Store at 15° to 30° in airtight containers.

Proprietary Preparations

Efudix (Roche, UK). Fluorouracil, available as a cream containing 5%. (Also available as Efudix in Austral., Belg., fr., Ger., Ital., Jap., Neth., S.Afr., Spain, Switz.).

Fluoro-uracil (Roche, UK). Fluorouracil sodium, available as **Capsules** each containing the equivalent of fluorouracil 250 mg, and as a solution containing the equivalent of fluorouracil 25 mg per ml, in **Ampoules** of 10 ml.

POLYMER PROFILE

HYDROXPROPYL METHYL CELLULOSE

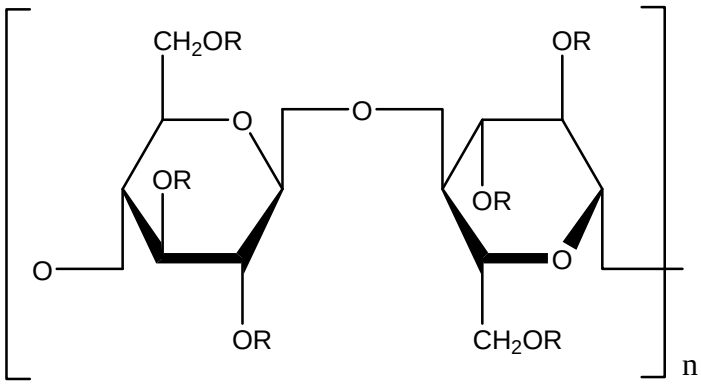
Synonym **:** Methocel, Hypromellose, Hypromellosum

Chemical name **:** Cellulose -2 hydroxypropyl methyl ether

Empirical formula **:** o-methylated and 0 – (2-hydroxpropylated) cellulose.

Molecular weight **:** 10,000 – 1,500,000

MOLECULAR STRUCTURE



Where R is H, CH_3, or $CH_3CH(OH)CH_2$

FUNCTIONAL CATEGORY:

Coating agent, film former, rate controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder, viscosity increasing agent.

APPLICATION IN PHARMACEUTICAL FORMULATION AND TECHNOLOGY:

Hypromellose is primarily used as a tablet binder (2% - 5% w/w concentration), film coating (2% - 20% w/w concentration), and as extended release matrix (10 – 80% w/w in formulation)

Hypromellose is used as a suspending[153] and thickening agent in topical formulation, particular ophthalmic preparation (0.45 – 1.0% w/w)

Hypromellose is also used as an emulsifier, suspending agent and stabilizing agent in topical gels and ointments. In addition hypromellose is used in the manufacture, as an adhesion in plastic bandages and as a wetting agent for hard contact lenses. It is also added is cosmetic and food products

DESCRIPTION:

Odourless, tasteless, white (or) creamy white fibrous (or) granular powder.

PHARMACEUTICAL SPECIFICATION:

pH	**:**	5.5 – 8.0 (1% w/w solution)
Melting point	**:**	$190^0 – 200^0$
Moisture content	**:**	Hypromellse absorbs moisture from the atmosphere.
Specific gravity	**:**	1- 26
Solubility	**:**	Soluble in cold water, forming a viscous colloidal solution. Insoluble in chloroform, ethanol and ether, but soluble in mixture of ethanol and dichloromethane, mixture of methanol and dichloro methane.

Table 3.1: Viscosity: A code range of viscosity types are commercial available in **(2% w/v) of aqueous solution)**

HYPROMELLOSE GRADE	VISCOSITY (mpas)
K 600 LVP	80 – 120
K 4 M	3000 – 5600
K 15 MP	12000 - 21000
K 100 MP	80000 - 120000
E 4 MP	3500 – 5600
E 10 MPCR	8000 – 13000
E 3 PREM LV	2.4 – 3.6
E 5 PREM LV	4 – 6
E 6 PREM LV	5 – 7
E 15 PREM LV	12 – 18
E 50 PREM LV	40 – 60
K 3PREM LV	2.4 - 3.6

STABILITY AND STORAGE CONDITIONS:

Hypromellose powder is a stable material although it is hygroscopic after drying. Solutions are stable at pH – 3-11. Aqueous solutions are comparatively enzyme resistant providing good viscosity stability during long-term storage. Hypromellose powder should be stored in a well closed contains in a cool, dry place.

POLYMETHACRYLATES

NONPROPRIETARY NAMES

USP – Ammonio methacrylase copolymer

USP – Methacrylic acid copolymer

SYNONYMS

Eudragit ; Kollicoat ; Polymeric methacrylates

COMMERCIAL FORM

- Eudragit RL 100 / Eudragit RS 100
- Eudragit RLPO / Eudragit RSPO

Solid substances obtained from Eudragit RL100 or Eudragit RS100.

FUNCTIONAL CATEGORY

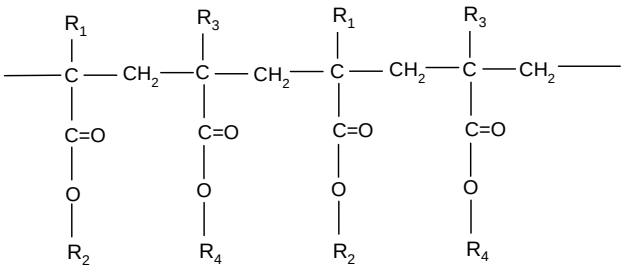
Film former; Tablet binder

EXPLANATION

Eudragit RL100 (Tape A) / Eudragit RS100 (Type B) and Eudragit RLPO (Type A / Eudragit RSPO (Type B) are copolymers synthesized[154] from acrylic acid and methacrylic acid esters. With Eudragit RL (Type A) having 10% of functional quaternary ammonium groups and Eudragit RS (Type B) having 5% of functional quaternary ammonium groups. The ammonium groups are present as salts and make the polymers permeable.

Since quaternary ammonium groups determine the swelling ability of the films in water and their permeability to dissolved salts and medicinal substances, Eudragit RL, which contains more of these groups, forms highly permeable films with little delaying action. By contrast and owing to the reduced content in quaternary ammonium groups,

films of Eudragit RS swell less easily and are only slightly permeable to active ingredients.



For Eudragit RL and RS

R_1 = H, CH_3

R_2 = CH_3, C_2H_5

R_3 = CH_3

R_4 = $CH_2CH_2\ N(CH_3)_3^+\ Cl^-$

Average molecular weight is approx. 150000

Chemical name	Trade name
Poly (ethyl acrylate, methyl methacrylate, trimethyl ammonio ethyl methacrylate chloride)1:2:0.2	Eudragit RL 100 Eudragit RLPO
Poly (ethyl acrylate, methyl methacrylate, trimethyl ammonio ethyl methacrylate chloride)1:2:0.1	Eudragit RS 100 Eudragit RSPO

DESCRIPTION

Molecular weight = Approximately 150000

Appearance, Colour, Odour

- Eudragit RL 100 and Eudragit RS 100 – Colourless, clear to cloudy granules with a faint amine like odour.
- Eudragit RLPO and Eudragit RSPO – White powder with a faint amine like odour.

Solubility

- 1 gm of the substances dissolves in 7 gm aqueous methanol, ethanol, isopropyl alcohol as well as in acetone, ethyl acetate and methylene chloride to give clear to cloudy solutions.
- The substances are practically insoluble in petroleum ether, 1N sodium hydroxide and water.

PHARMACOPEIAL SPECIFICATIONS

Table3.2: Specification for ammonio methacrylate copolymers (Eudragit RL and RS)

Test	USP
Identification	+
Viscosity	15 mPa S
Loss on drying	3.0%
Residue on ignition	0.1%
Arsenic	2 ppm
Heavy metals	0.002%
Monomers	0.15%
Assay of ammonio methacrylate units (dried basis) Type A Type B	 8.85 – 11.96% 4.48 – 6.77%

TYPICAL PROPERTIES

- Density (Bulk) - 0.39 g/cm^3
- Density (Tapped) - 0.424 g/cm^3

- Density (True) - 0.816 – 0.836 g/cm^3
- Refractive index

  n_D^{20} - 1.38 – 1.385
- Viscosity (Dynamic) - 15 mPaS

Table 3.3: Summary of Properties of Polymethacrylates

Type	Supply form	Polymer dry weight content	Recommended solvents	Characteristics	Application
Eudragit RL100	Granules	97%	Acetone, Alcohols	High permeability	Sustained Release
Eudragit RLPO	Powder	97%	Acetone, Alcohols	High permeability	Sustained Release
Eudragit RS100	Granules	97%	Acetone, Alcohols	Low permeability	Sustained Release
Eudragit RSPO	Powder	97%	Acetone, Alcohols	Low permeability	Sustained Release

4.1 Basis of Selection of Polymers (Polymethacrylates)

1. These polymers (Eudragit) are used only for oral sustained or controlled drug delivery system.
2. Polymethacrylates are only the polymers having different types with different permeable characteristics in the same group.

Example, Eudragit RL films are more permeable than those of Eudragit RS and by mixing the two types together films of varying permeability can be obtained.

3. Having the same solubility characteristics of the selected polymers, common solvent and common method of preparation can be used for preparation of microspheres.

4. Finally, they have common mechanism of release, i.e. diffusion.

In this study, 5 fluorouracil loaded microspheres were prepared by taking four different types of polymethacrylates, Eudragit RL 100, Eudragit RS 100, Eudragit RSPO and Eudragit RLPO

Eudragit RL and Eudragit RS have 10% and 5% of functional quaternary ammonium groups respectively. Quaternary ammonium groups determine the swelling ability of the films and their permeability to water, dissolved salts and medicinal substances. Eudragit RL, which contains more of these groups forms highly permeable films with little delaying action. By contrast, and owing to the reduced content in quaternary ammonium groups, films of Eudragit RS swell less easily and are only slightly permeable to active ingredients.

EXCIPIENT PROFILE

SODIUM BICARBONATE

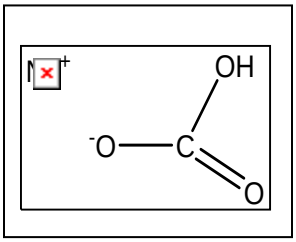
Synonyms : Baking soda, sodium hydrogen carbonate, Natrii, hydrogenocarbons

Chemical Name : Carbonic acid monosodium salt

Empirical formula : $NaHCo_3$

Molecular weight : 84.01

Structural Formula



Functional Category : Alkalizing agent, therapeutic agent

Application in pharmaceutical formulation:

Sodium bicarbonate is generally used in pharmaceutical formulation as a source of carbon dioxide in effervescent tablets and granules. It is also widely used to produce (or) maintain an alkaline pH in the preparation. In effervescent tablets and granules, sodium bicarbonate is usually formulated with citric and / or tartaric acid.

Tablets may also be prepared with sodium bicarbonate alone since the acid of gastric fluid is sufficient to cause effervescent and disintegration. It is also used in solution as a buffering agent.

Recently sodium bicarbonate had been used as a gas forming agent in floating controlled release oral dosage forms and in alginate raft systems.

Sodium bicarbonate is used as an antacid and as a source of bicarbonate anion in the treatment of metabolic acidosis. It is also used as a freeze drying stabilizer and in tooth pastes.

CONCENTRATION OF SODIUM CARBONATE USED

USE	**CONCENTRATION (%)**
Buffer in tablets	10 – 40
Effervescent tablets	25 – 50
Isotonic injection infusion	1.39

DESCRIPTION:

Odorless, white, crystalline powder with a saline, slightly alkaline taste. The crystal structure is monoclinic prisms.

PHARMACEUTICAL SPECIFICATION:

pH – 8.3

Melting point – 270^0 (With decomposition)

Moisture content – Below 80% RH, the moisture content is less than 1% w/w

Solubility – At 20° 1 in 11 parts are soluble in cold water, practically insoluble in ethanol (95 %) and ether.

STABILITY AND STORAGE CONDITIONS

When heated to about 50° sodium bicarbonate begins to dissociate into carbon dioxide, sodium carbonate and water on heating to 250-300°, for a short time, sodium bicarbonate is completely converted into anhydrous sodium carbonate. Sodium bicarbonate powder is stable below 76 % relative humidity at 25° and below 48 % relative humidity at 40°. Sodium bicarbonate is stable in dry air but slowly decomposes in moist air and should therefore be stored in a well closed contain in a cool, dry place

INCOMPATIBILITIES:

Sodium bicarbonate reacts with acids, acidic salts, and many alkaloid salts with evolution of carbon dioxide. Sodium bicarbonate can also intensify the darkening of salicylates.

ISO PROPYL ALCOHOL

Synonyms **:** Dimethyl Carbinol

Chemical Name **:** Prapan -2-ol

Empirical formula **:** C_3H_80

Molecular weight **:** 60.1

Structural Formula **:** $(CH_3)_2CHOH$

Description **:** Clear, colorless, mobile, volatileliquid with a characteristics spirituous odour, resembling that a mixture of ethanol &acetone, it has slightly bitter taste**.**

Solublity **:** It is miscible with benzene, chloform, ethanol ether, glycerin& water. It is soluble in acetone, Insoluble in salt solutions.

Stablity **:** Stored in air tight container in a cool dry place.

Incompatibilities:

It is incompatible with oxidizing agents such as a hydrogen peroxide and nitric acid, which causes decomposition. IPA may be salted out from aqueous mixtures by the addition of sodium chloride, sodium sulphide and other salts or the addition of sodium hydroxide.

LACTOSE

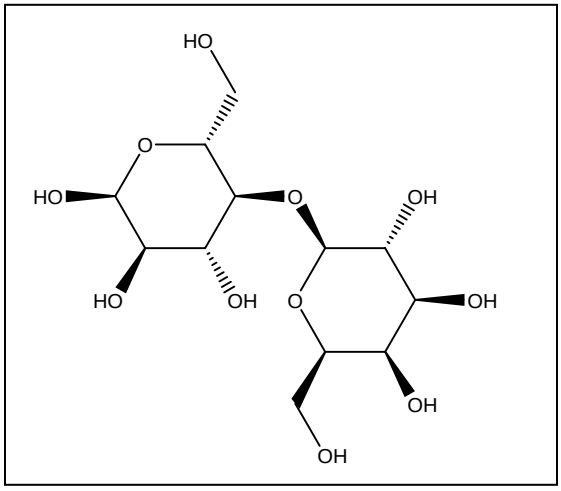
Synonyms: Lactose monohydrate, Lactosum,

Chemical Name: O-β - D- Galactopyranosyl (1-4)d – D- glucopyranose anhydrous

Empirical Formula: C_{12} H_{22} 0_{11}

Molecular Weight: 342.30

Structural Formula:



Functional Category: Tablet and capsule diluent

Application in pharmaceutical formulation

Lactose is widely used as a filler (or) diluent in tablet, capsules, and to a limited extent in lyophilized products and infant feed formulations. Spray dried lactose was used in solid dosage pharmaceutical formulation. Direct compression grades of lactose are often used to carry small quantities of drug and this permits tablets to make without granulating. Concentration of lactose generally used in formulation was up to 85%. The application of lactose include as a carrier (or) diluent for inhalation products and in lyophilized product. Where lactose is added to freeze dried solutions to increase plug size and aid caking. Lactose is used in the combination with sucrose to prepare sugarcoating solutions.

Description:

White crystalline particles (or) powder. It is odorless and slightly sweet tasting.

Pharmaceutical specification

Melting point – 201 to 202° for lactose monohydrate 252.2° for anhydrous β - lactose

Solubility:

At 25° 1 in 4.63 parts are soluble in water, practically insoluble in chloroform, ethanol, ether, water.

Stability and storage conditions:

Under humid condition mold growth may occur. Lactose may develop a brown coloration on storage, the reaction being accelerated by warm, dry condition. Lactose should be stored in a well-closed container in a cool, dry, place.

Incompatibilities:

A Millard type of condensation is likely to occur between lactose and compound with primary amine group to form brown colored products. Lactose is incompatible with amino acid, amino phylline and amphetamine

POLYVINYL PYROLIDONE (PVP K30)

Polyvinyl pyrrolidone[153] is a white to creamy white odorless, hygroscopic powder. It is used as tablet blender, suspending agent or viscosity increasing agent. In tableting, polyvinyl pyrrolidone solutions are used as binders in wet granulation process. PVP is also added to powder blends in the dry form and granulated to situ by the addition of water, alcohol polyvinyl pyrolidone solutions may also be used as coating agent. It is incomplete in solution with a wide range of inorganic salts, natural and synthetic resins & other chemicals. The efficacy of some preservatives e.g., Thiomersal may adversely affected by the formulation of complexes with PVP.

MAGNESIUM STEARATE

Magnesium stearate is a fine white precipitated or milled powder, odour and taste are sight but characteristic. The powder is unctuous and really adheres to skin. T is insoluble in water, alcohol and ether slightly soluble in hot alcohol and benzene.

It is used as tablet capsules lubricant, glidant or anti adherent in the large of 0.5 to 2.0%. Due to its hydrophobic nature, it may retard the dissolution of a drug from solid dosage form. It is non-stable, non-self polymerizable and should be stored in a cool & dry place and in a well-closed container. It is in compatible with strong acid, alkalis and iron salts.

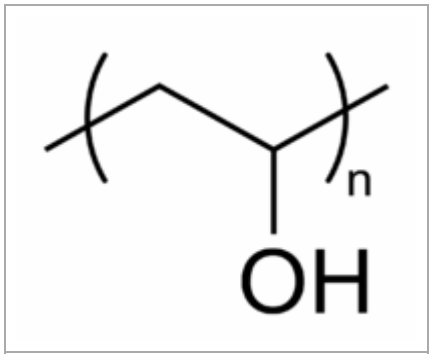
TALC

Talc is very fine, white to grayish. White odourless crystalline powder, unctuous adheres readily to skin, soft to touch & free from grittiness it is used an tablet and capsules lubricant, glidant and or anti caking agent chemically it is hydrous magnesium silicate may contain a small amount of aluminum silicate.

1-4% of the talc used as lubricant or glidant in tablets and manufacture 5-0% of talc is used as fuller fro tablets and capsule. 90 of talc is used a dusting powder, both release rate and release profile can be adjusted by incorporation of talc as a hydrophobic excipient. It the incompatible wish quaternary ammonium compounds. It is stable, preserved in well-closed container. It should not be inhaled practically non-toxic on ingestion. Prolonged and intense exposure to talc may produce pneumoconiosis.

POLYVINYL ALCOHOL

Polyvinyl alcohol (**PVOH**, **PVA**, or **PVAL**) is a water-soluble synthetic polymer



Properties

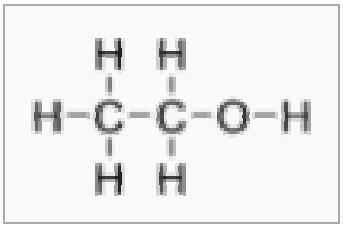
Molecular formula	$(C_2H_4O)_x$
Density	1.19-1.31 g/cm³
Melting point	230°C
Boiling point	228°C

Properties

Polyvinyl alcohol has excellent film forming, emulsifying, and adhesive properties. It is also resistant to oil, grease and solvent. It is odorless and nontoxic. It has high tensile strength and flexibility, as well as high oxygen and aroma barrier properties. However these properties are dependent on humidity, in other words, with higher humidity more water is absorbed. The water, which acts as a plasticiser, will then reduce its tensile strength, but increase its elongation and tear strength. PVA is fully degradable and is a quick dissolver. PVA has a melting point of 230°C and 180–190°C for the fully hydrolysed and partially hydrolysed grades. It decomposes rapidly above 200°C as it can undergo pyrolysis at high temperatures.

PVA is an atactic material but exhibits crystallinity as the hydroxyl groups are small enough to fit into the lattice without disrupting it.

ETHANOL



Ethanol is a straight-chain alcohol, and its molecular formula is C_2H_5OH. Its empirical formula is C_2H_6O. An alternative notation is CH_3-CH_2-OH, which indicates that the carbon of a methyl group (CH_3-) is attached to the carbon of a methylene group (-CH_2-), which is attached to the oxygen of a hydroxyl group (-OH). It is a constitutional isomer

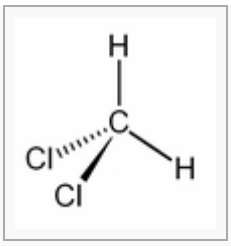
of dimethyl ether. Ethanol is often abbreviated as **EtOH**, using the common organic chemistry notation of representing the ethyl group (C_2H_5) with **Et**.

The hydroxyl group generally makes the alcohol molecule polar. Those groups can form hydrogen bonds to one another and to other compounds. This hydrogen bonding means that alcohols can be used as protic solvents. Two opposing solubility trends in alcohols are: the tendency of the polar OH to promote solubility in water, and of the carbon chain to resist it. Alcohols, like water, can show either acidic or basic properties at the O-H group. With a pK_a of around 16-19 they are generally slightly weaker acids than water

Alcohols have applications in industry and science as reagents or solvents. Because of its low toxicity and ability to dissolve non-polar substances, ethanol can be used as a solvent in medical drugs, perfumes, and vegetable essences such as vanilla. In organic synthesis, alcohols serve as versatile intermediates.

Ethanol can be used as an antiseptic to disinfect the skin before injections are given, often along with iodine. Alcohol is also used as a preservative for specimens.

Dichloromethane



Dichloromethane (**DCM**) or **methylene chloride** is the chemical compound with the formula CH_2Cl_2. It is a colorless, volatile liquid with a moderately sweet aroma. It is widely used as a solvent, the general view being that it is one of the less harmful of the chlorocarbons. Although it is not miscible with water, it will dissolve in most organic solvents Dichloromethane's volatility and ability to dissolve a wide range of organic compounds makes it an ideal solvent for many chemical processes

PREFORMULATION STUDIES

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone. It is the first step in the rational development of dosage form.

MATERIALS AND METHODS

Analysis of 5 fluoro uracil

◊ **By U.V Spectroscopy**

Absorbance of a 0.002% w/v solution in a mixture of 99 volumes of methanol and 1 volume of 1M hydrochloric acid at the maximum at about 266 nm and at the minimum of about 232 nm and was represented in Fig no 4.1.

◊ **By I.R Spectroscopy**

5 fluoro uracil discs were prepared by pressing the 5 fluoro uracil with potassium bromide and the spectra between 4000^{-1}cm – 500^{-1}cm was obtained under the standard operational conditions. The absorption maxima in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented in Fig.4.2 respectively.

◊ **Melting Point**

Melting point of the drug was determined by capillary tube method and the value is shown in Table: 4.1

◊ **Loss on Drying**

The sample was dried at a pressure below 5mm of Hg for 3 h and weight loss on drying was determined[155] and the value is shown in Table: 4.1

◊ **Determination of Bulk and Tapped Density**

An accurately weighed quantity of the powder (w), which was previously passed through # 40 was carefully poured into the graduated cylinder and the volume (v_o) was measured. The graduated measuring cylinder was tapped for 100 times and after that, the volume (v_f) was measured and continued the operation till the two

consecutive readings were equal. Bulk density and tapped density determines the floating capacity of the formulation. The bulk density and tapped density were calculated using the formulas and the value is shown in Table: 4.1.

Bulk density = w/v_o

Tapped density=w/v_f

◊ **Compressibility Index**

Compressibility is an important measure that can be obtained from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is. A material having values of less than 20 to 30% is defined as the free flowing material[156]. The compressibility of the powder was calculated by determining the Carr's index and value are shown in Table: 4.1

Carr's index % = $100(v_o-v_f)/v_o$

◊ **Angle of Repose**

The angle of repose represents flow property of powder. The angle of repose can be determined by fixed funnel method and the value is given in Table: 4.1. In this method, a funnel was clamped with its tip at a required height, above a graph paper, that was placed on a flat horizontal surface The powder was allowed to flow carefully through the funnel until apex of the conical pile just touches the tip of the funnel.[157] The radius of the pile was determined .The angle of repose was calculated as

$$\tan \theta = h/r$$

$$\theta = \tan^{-1} h/r$$

Where, θ - Angle of repose

h - Height of the pile

r - Radius of the pile

◊ **Residue on Ignition**

Weighed, accurately 2g of 5 fluoro uracil in a crucible that was previously ignited cooled and weighed. Heated gently at first, until the substance was thoroughly

charred, cooled and then moistened the residue with 2ml of nitric acid and sulphuric acid until carbon was consumed. Cooled in dessicator, weighed and the percentage of residue was calculated and the value is shown in Table: 4.1

Drug Excipients compatibility study

Sample Preparation – Each excipients used in the formulations was blended with the drug level that are realistic with respect to the final dosage form. Each excipient was thoroughly blended with drug to increase drug-excipient molecular contacts and to accelerate the reactions if possible. Each drug excipients blend was taken separately into vials and kept for one month and two months study at 40° C. After that, each blend was tested for stability by physical observation. Results are tabulated in Table: 4.2

Drug Excipients compatibility study by I.R Spectroscopy

Potassium bromide discs were prepared by pressing the 5 fluoro uracil along with excipients and the spectra between 4000^{-1}cm – 500^{-1}cm was obtained under the operational conditions. The absorption maxima in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented in Fig.4.2- 4.16 respectively.

PREPARATION OF STANDARD GRAPH OF 5 FLUORO URACIL

Preparation of simulated gastric fluid (SGF) pH 1.2

50 ml of 0.2M potassium chloride was placed in a 200 ml volumetric flask and the specified volume of 0.2M HCL(pH 1.2) was added and the final volume was made up to 1000ml with distilled water.

Standard curve for 5 fluoro uracil in simulated gastric fluid

100mg of pure 5 fluoro uracil was accurately weighed and transferred to 100 ml volumetric flask and volume was made up to 100 ml with SGF pH 1.2. Serial dilution of 1µg -10µg was made. The absorbance was measured at 266 nm by using UV spectrophotometer. The values are given in Table 4.3 and represented in Fig. 4.17 respectively.

RESULTS AND DISCUSSION

The physical properties of the drug substance, 5 fluoro uracil and compatibility study of the drug substance with various excipients of different formulations were investigated.

The drug was identified by U.V spectra and IR.spectra. The results were presented in the Fig 4.1and 4.2 and it coincides with standard spectra. The physical properties such as melting point, loss on drying, bulk and tapped density, compressibility index, angle of repose, residue on ignition was determined and results were given in the table 4.1. The results were found to satisfy the USP specifications. Each excipient used in the development of formulation was blended with the drug approximately in the same ratio used in the formulation and stored at 40ºC for one month and two month period and the observation are shown in the table 4.2. The drug excipient compatibility observations revealed that the drug blended with other excipients has no change in color. Drug excipients and polymer interaction was also carried out by I.R studies as shown in Fig 4.2 – 4.16 respectively. IR study of drug excipient compatibility showed no interaction of drug with excipients under study.

Standard dilutions for the drug 5 FU, in SGF medium were prepared and analyzed spectrophotometrically at 266 nm. The values are presented in the Table 4.3. The calibration curve is shown in Fig17. The standard graphs were used for the estimation of 5 FU GRS (floating) tablets for drug content and in vitro dissolution studies. The correlation coefficient of best fit line was 0.9990 and regression coefficient was found to be 0.9994.

Table: 4.1. Physical Characteristics of Drug (5 Fluoro Uracil)

S.No	Parameter	Specifications
1	Loss on Drying (%)	0.60
2	Bulk density (g/cc)	0.465
3	Tapped Density (g/cc)	0.593
4	Compressibility index (%)	21.68
5	Angle of repose (°)	39°71'
6	Melting point (° C)	230
7	Residue on ignition	0.12

Table: 4.2. Drug Excipients Compatibility Study

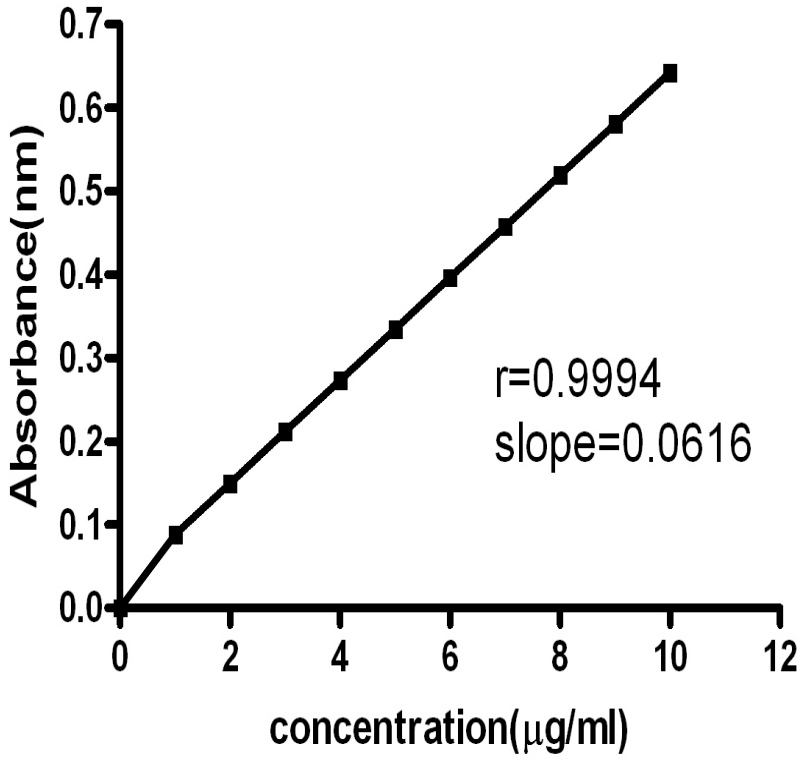
S.No	Drug +Excipients (Ratio: 1:1)	Observation				
		First month			Second month	
		Initial	RT	40^0C	RT	40^0C
1	D+HPMC K100	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour
2	D+HPMC KV600	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour
3	D+HPMC K4M	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour
4	D+HPMC K50	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour
5	D+PEG 6000	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour
6	D+Sodium Bicarbonate	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour
7	D+PVP K30	No change in colour	No change in colour	No change in colour	No change in colour	No change in colour

RT – Room temperature, D – Drug

Table 4. 3: Calibration Data

Sl.no	Concentration(µg/ml)	Absorbance(nm)
0	0	0.0000
1	1	0.0882
2	2	0.1498
3	3	0.2114
4	4	0.2730
5	5	0.3346
6	6	0.3962
7	7	0.4578
8	8	0.5194
9	9	0.5810
10	10	0.6426

Fig 4.17: Calibration Graph



DRUG POLYMER COMPATIBILITY STUDY

Fig 4.2: IR spectra of 5 Fluorouracil

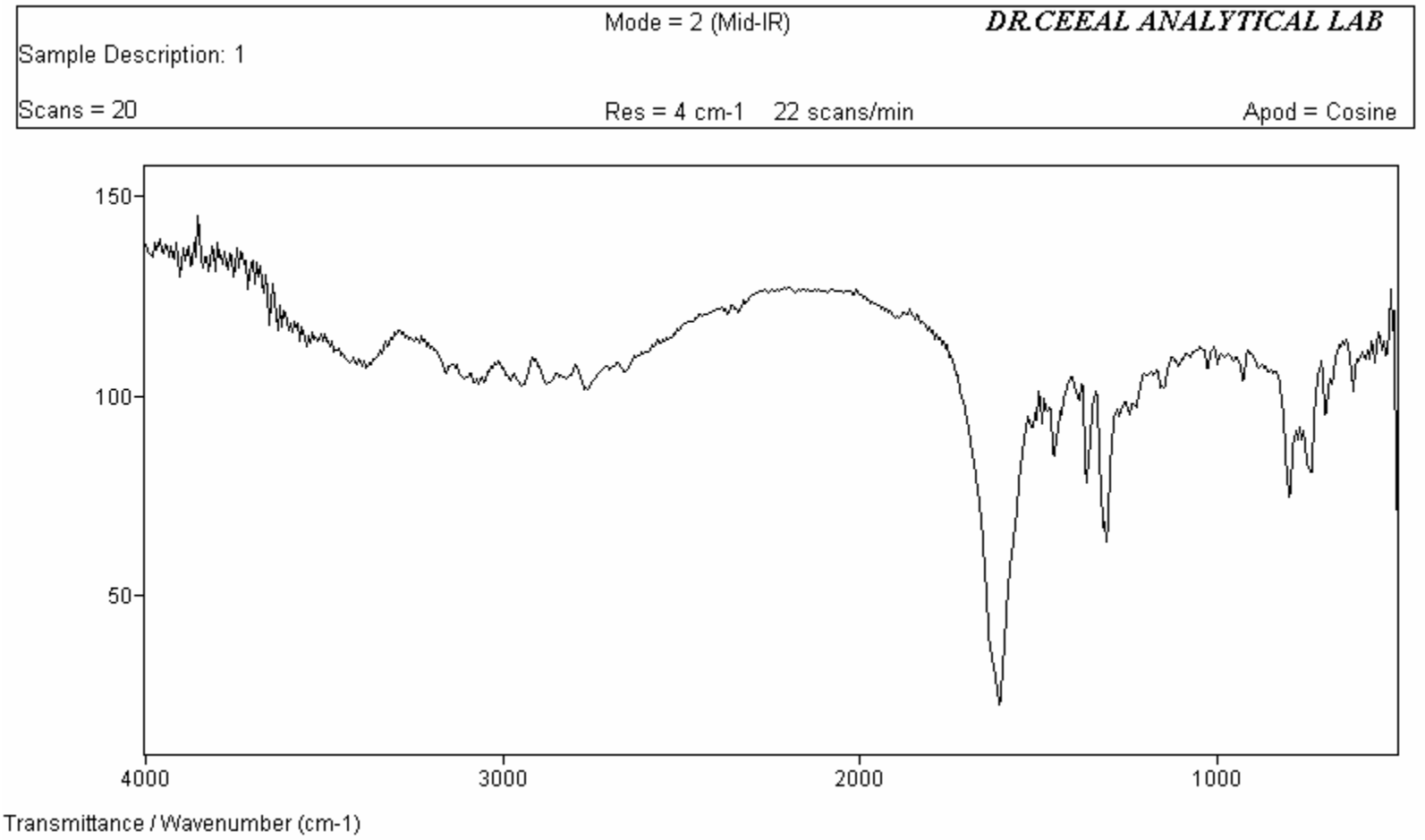


FIG 4.14: IR spectra of EUDRAGIT RSPO

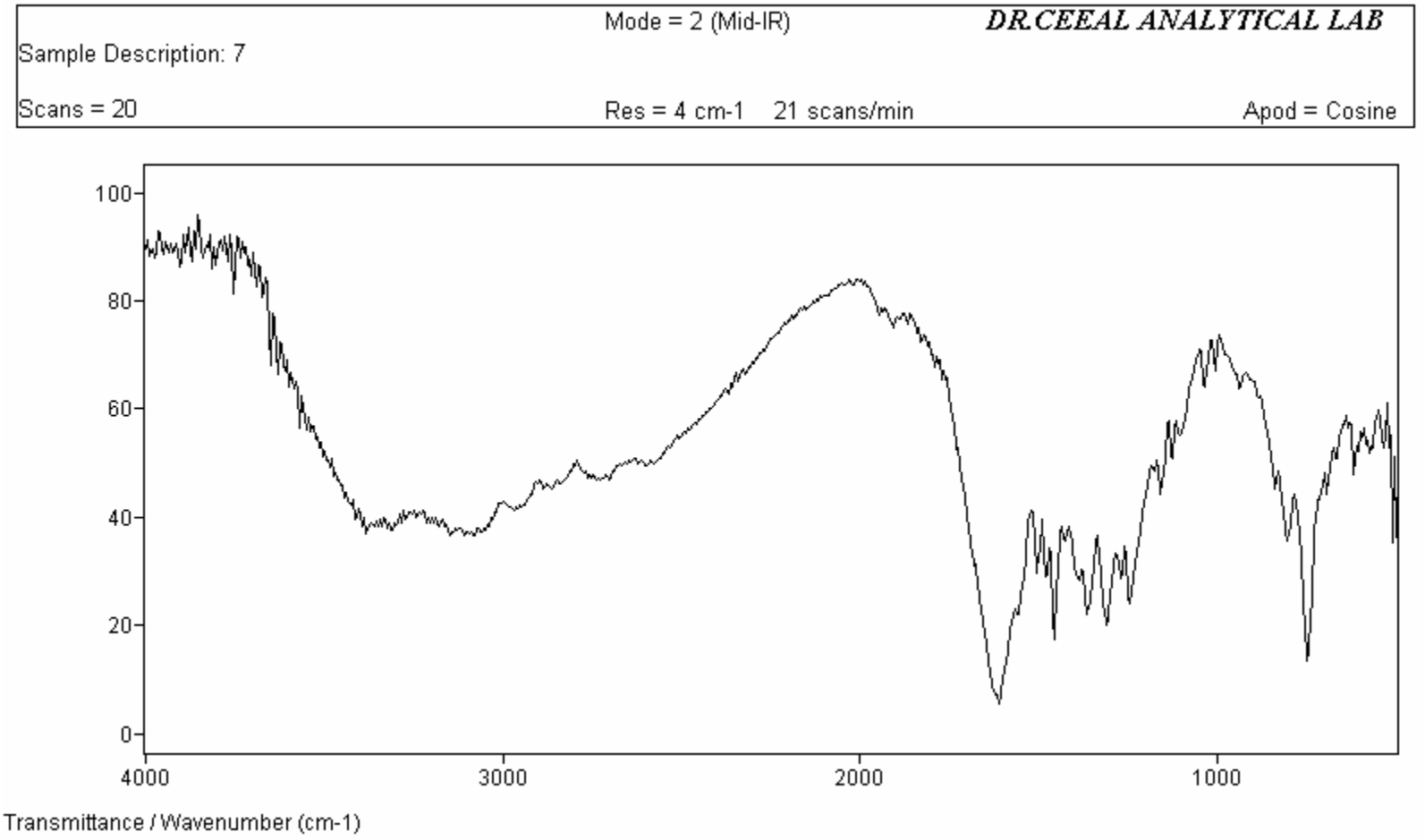


Fig 4.13: IR spectra of Eudragit RLPO

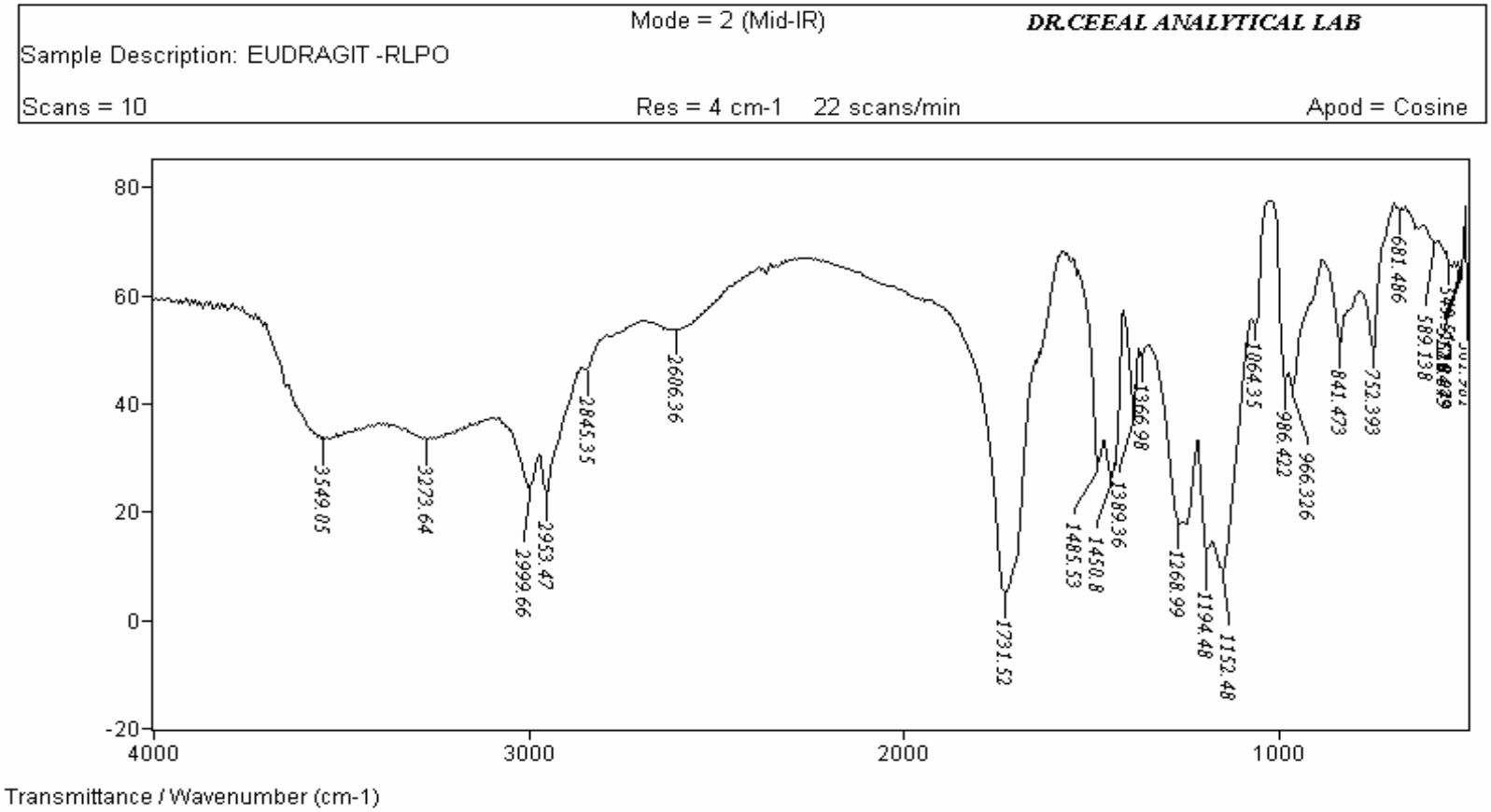


Fig 4.12: IR spectra of Eudragit RS 100

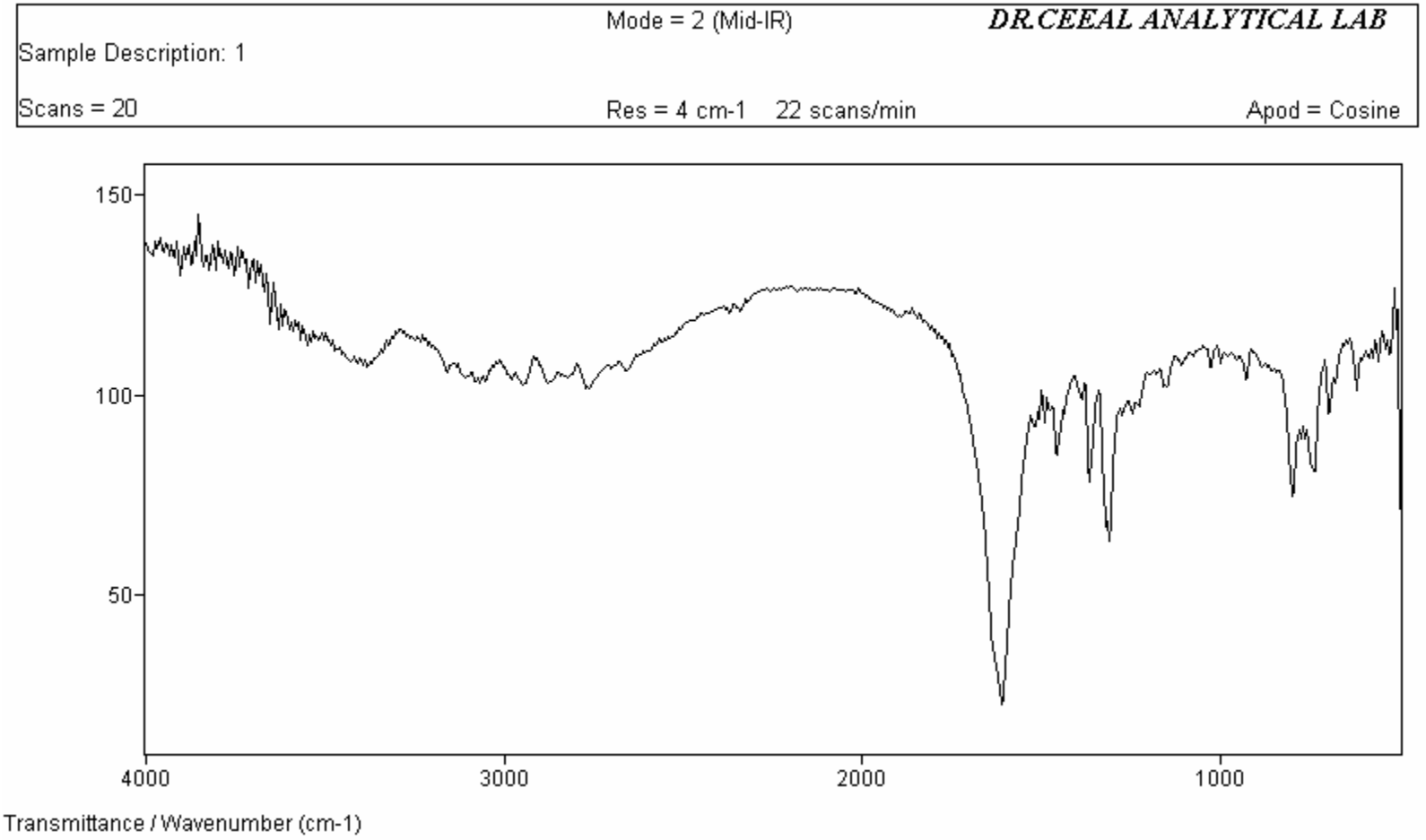


Fig 4.11: IR spectra of Eudragit RL 100

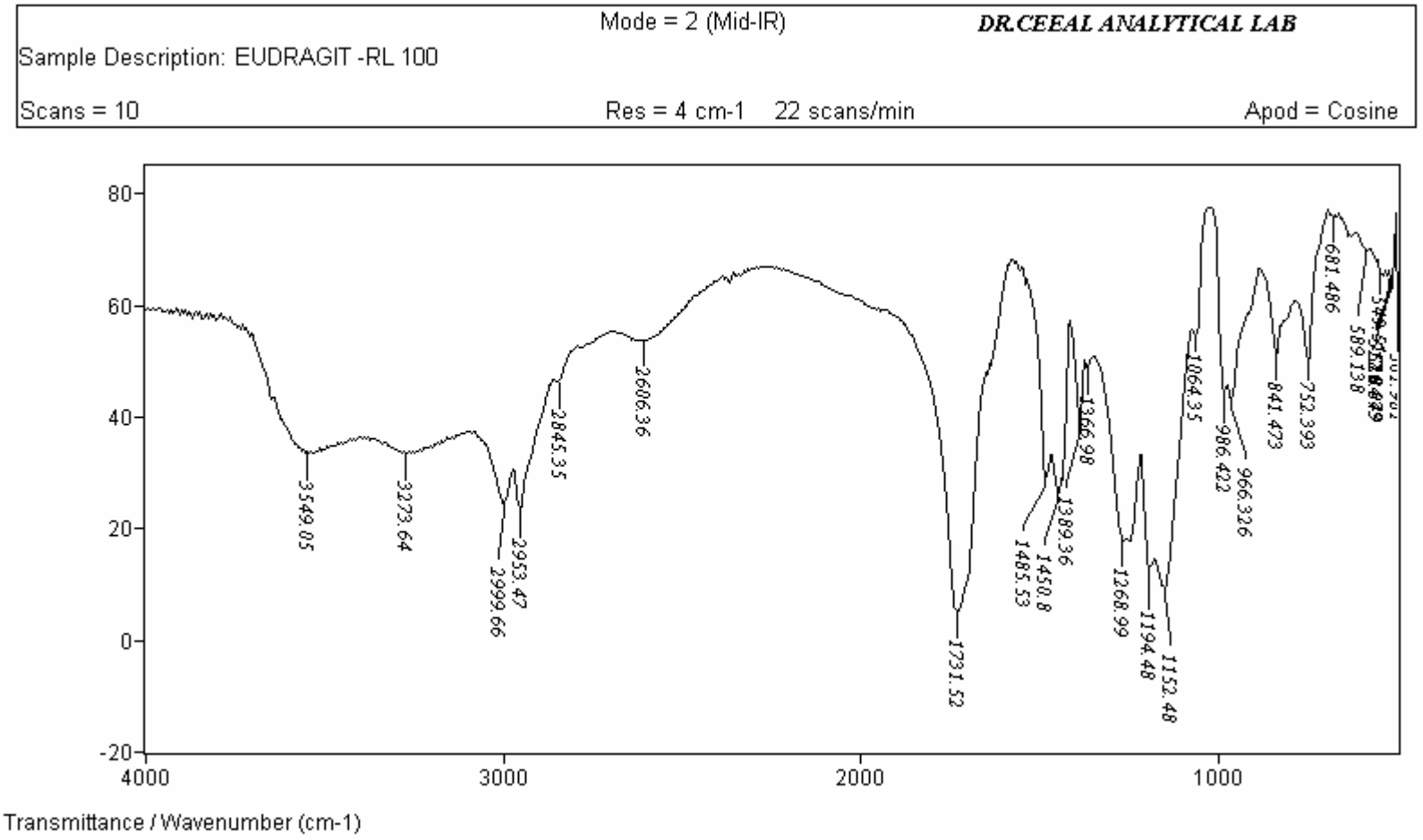


Fig 4.15: IR spectra of β Cyclodextrin

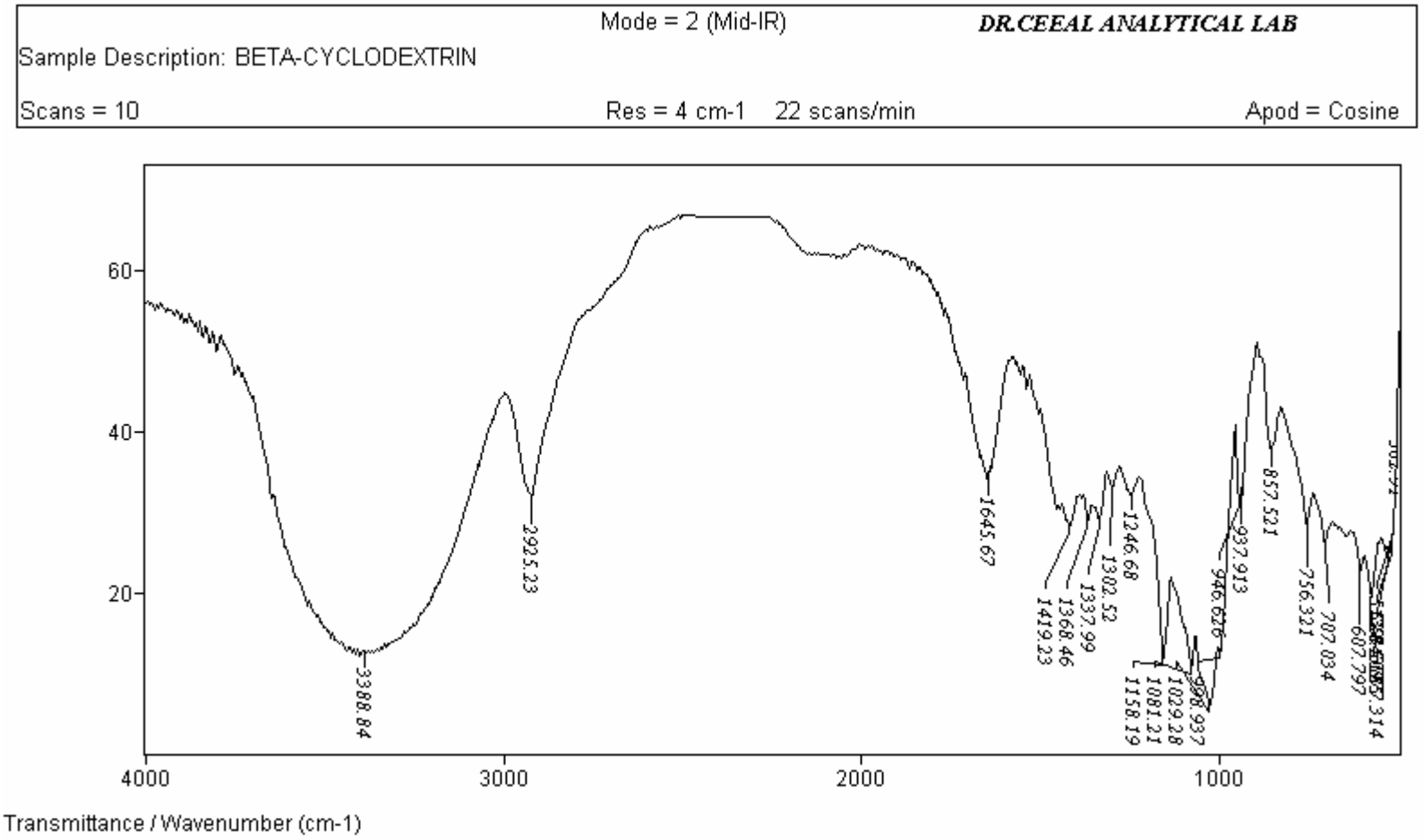


Fig 4.4: IR spectra of HPMC KV 600

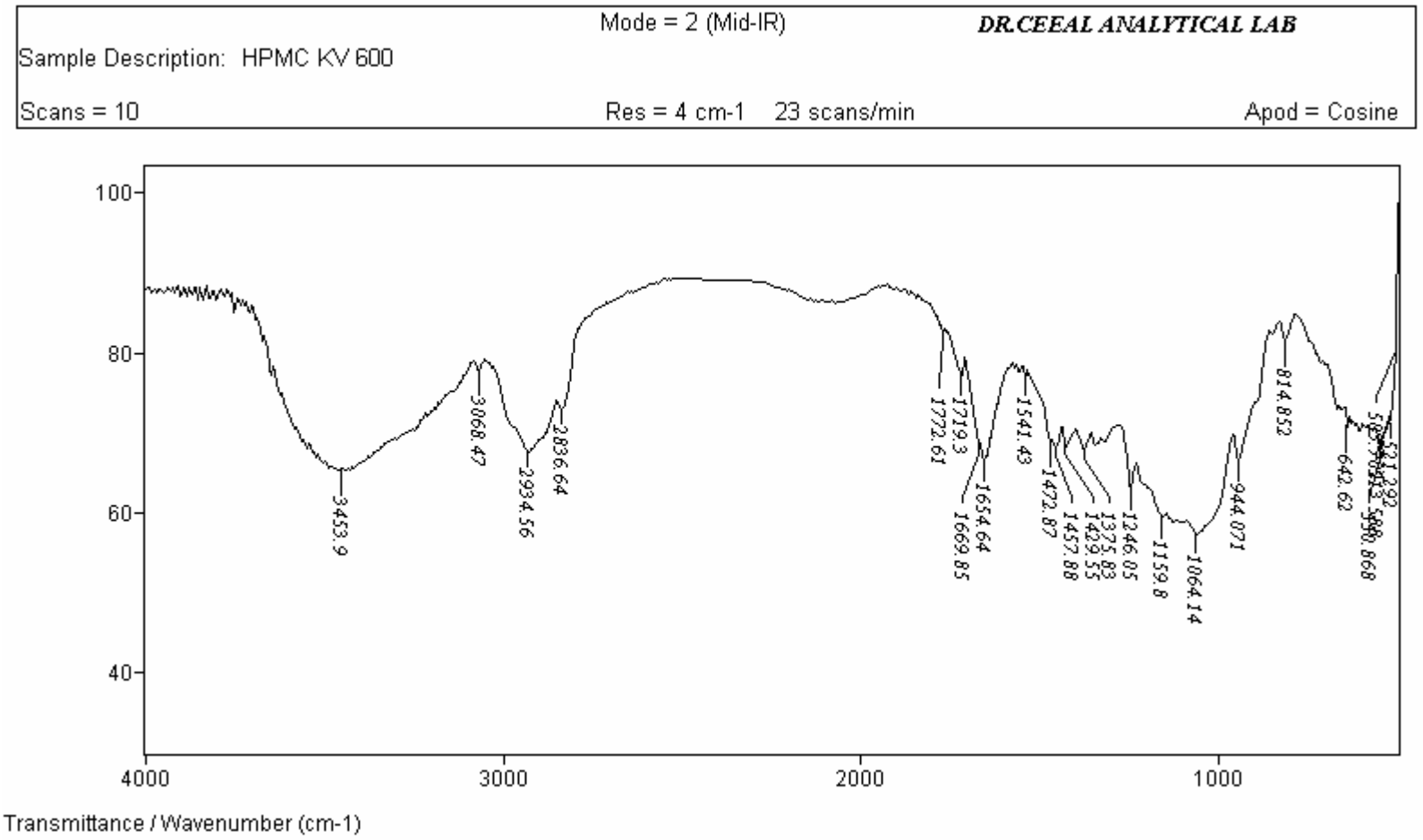


Fig 4.8: IR spectra of drug and HPMC KV 600

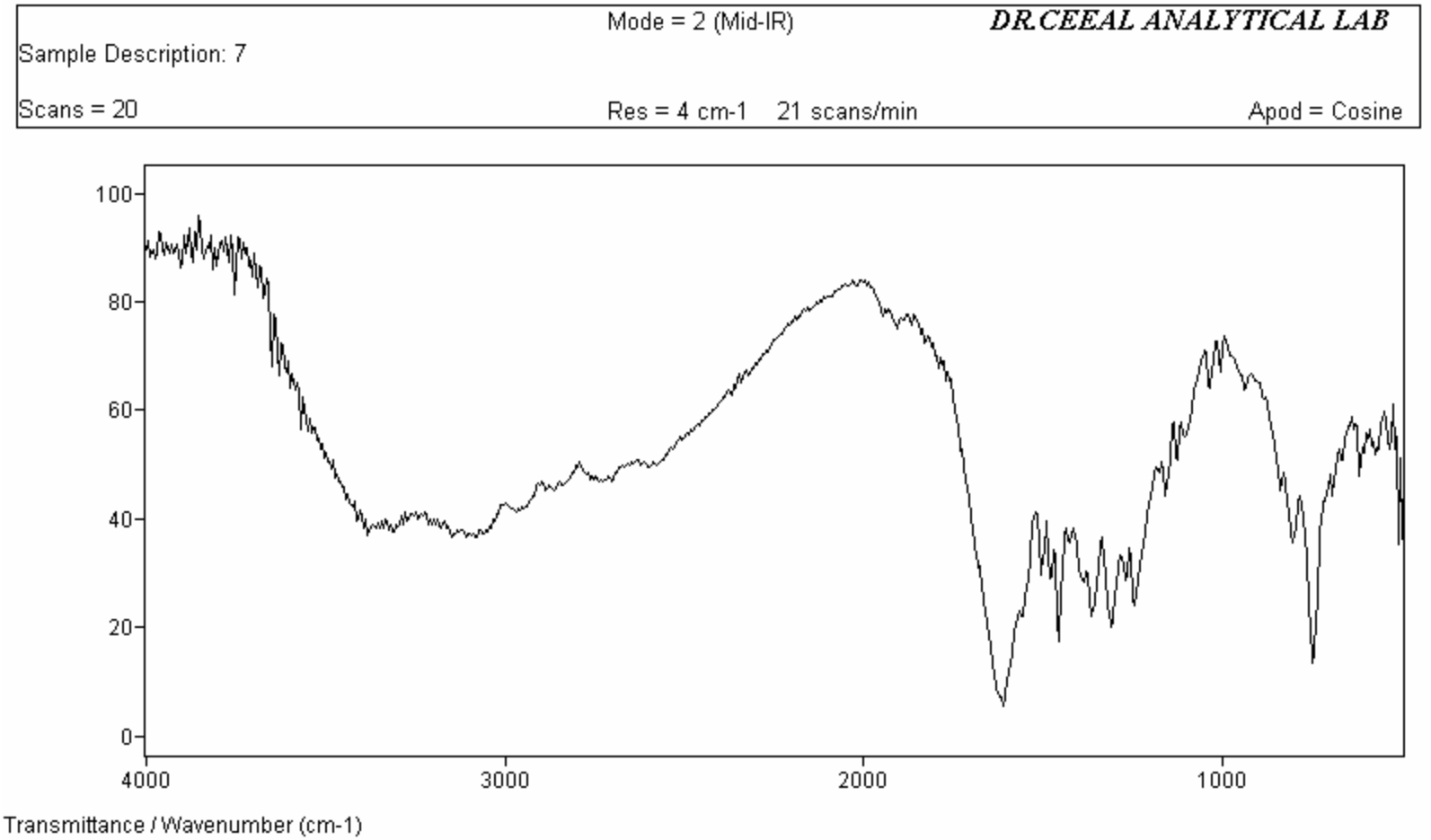


Fig 4.9: IR spectra of Drug and HPMC K50

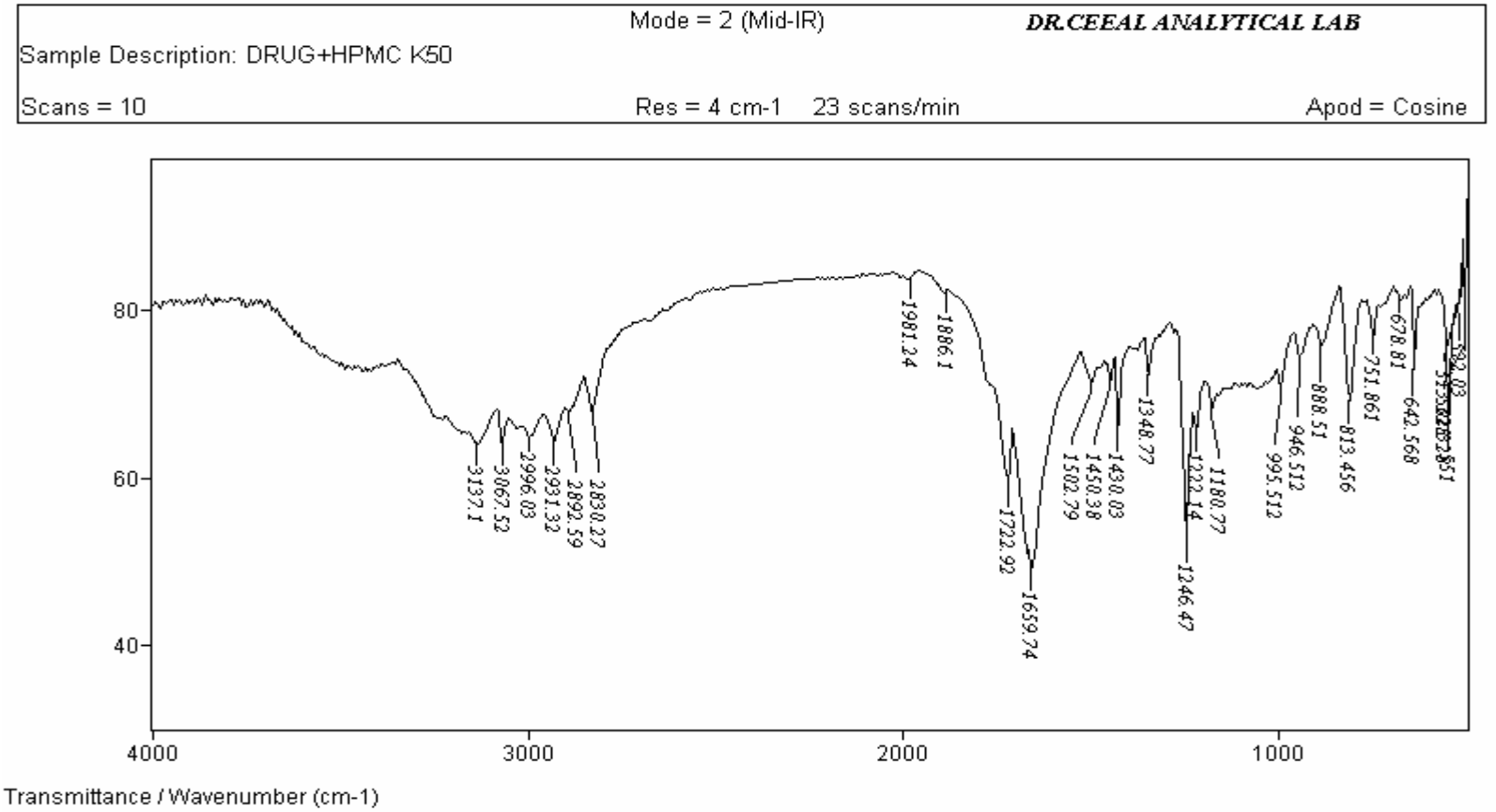


Fig 4.7: IR spectra of Drug and HPMC K100

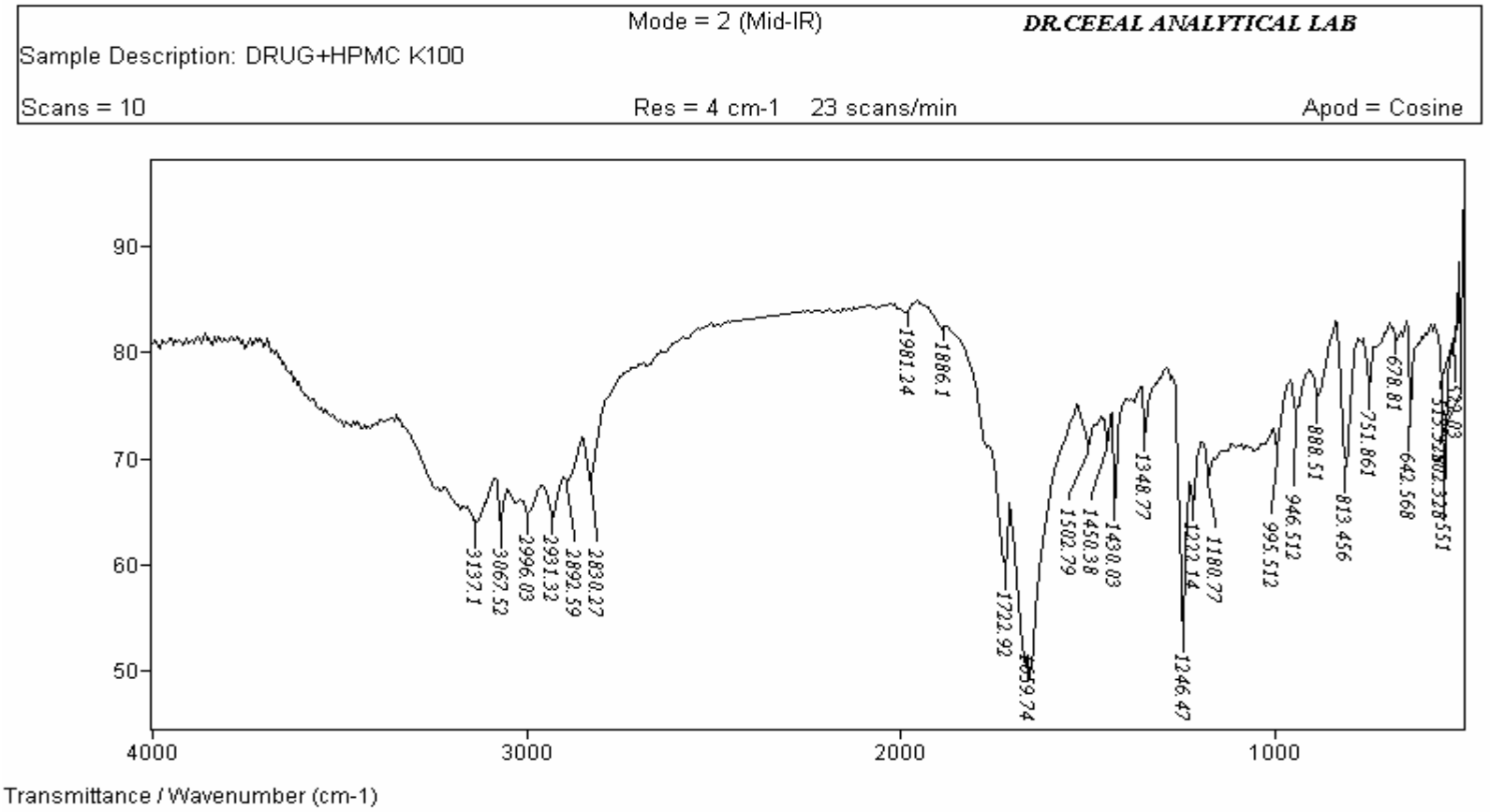
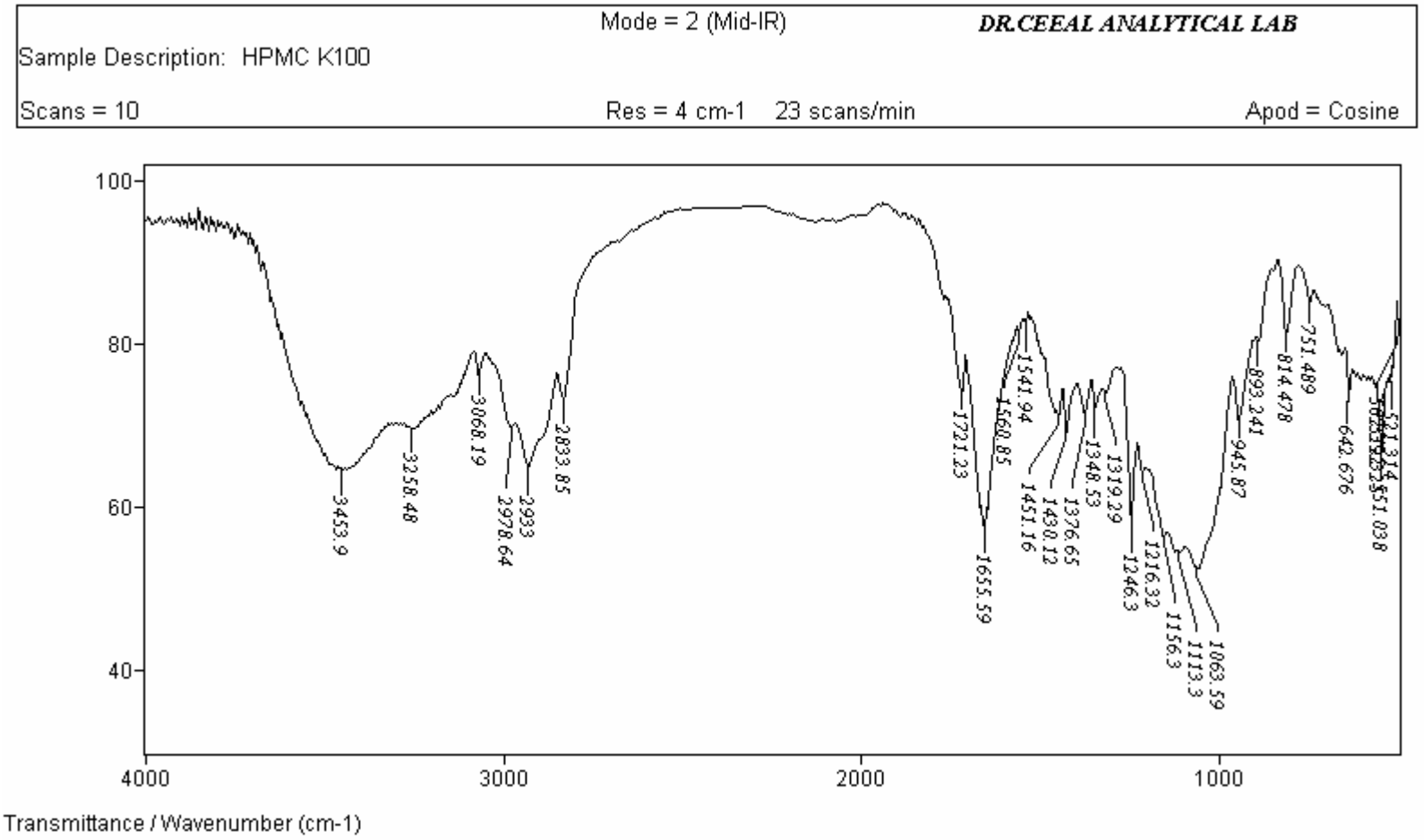


Fig 4.3: IR spectra of HPMC K 100



Transmittance / Wavenumber (cm-1)

Fig 4.5: IR spectra of HPMC K 50

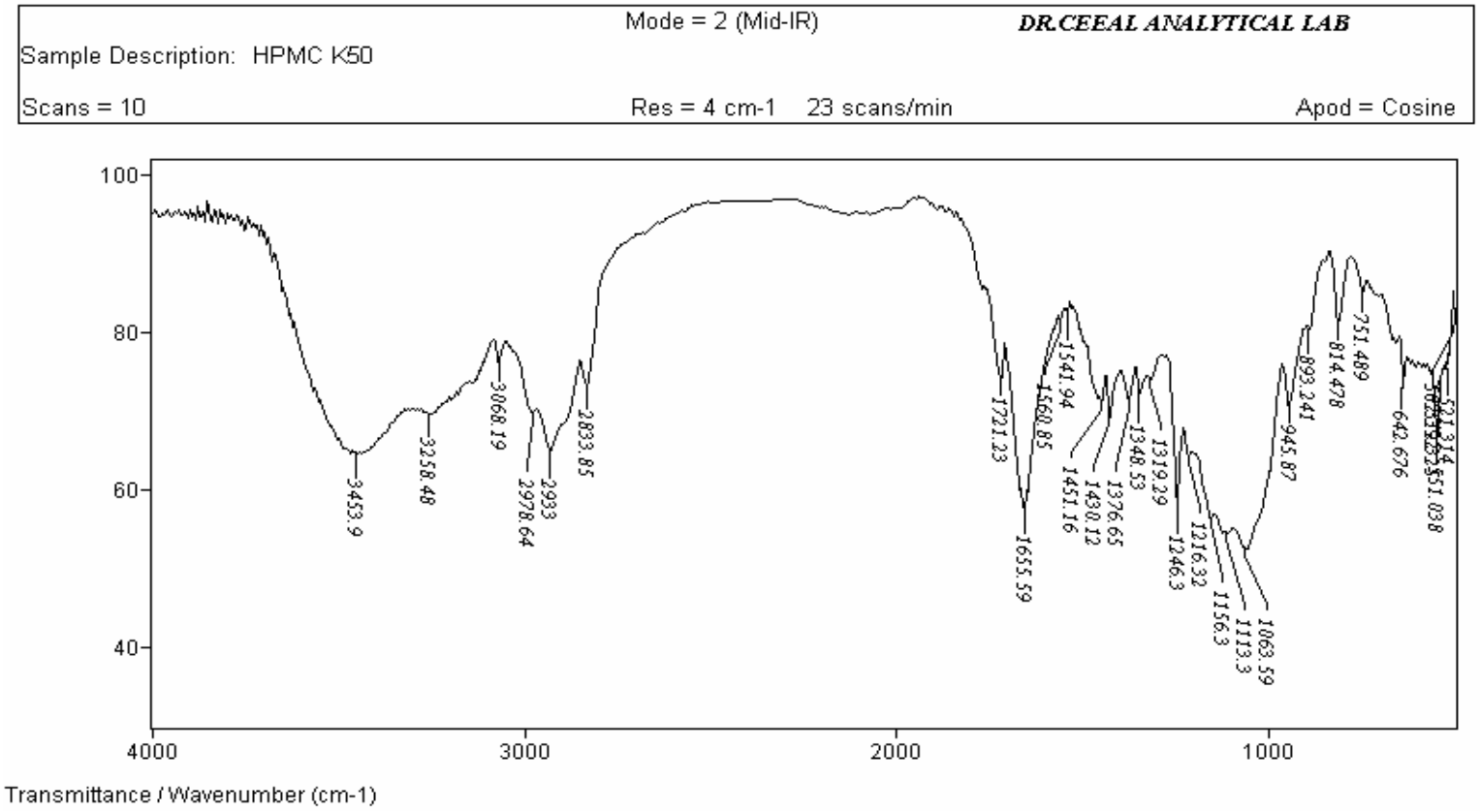


Fig 4.6: IR spectra of HPMC K4M

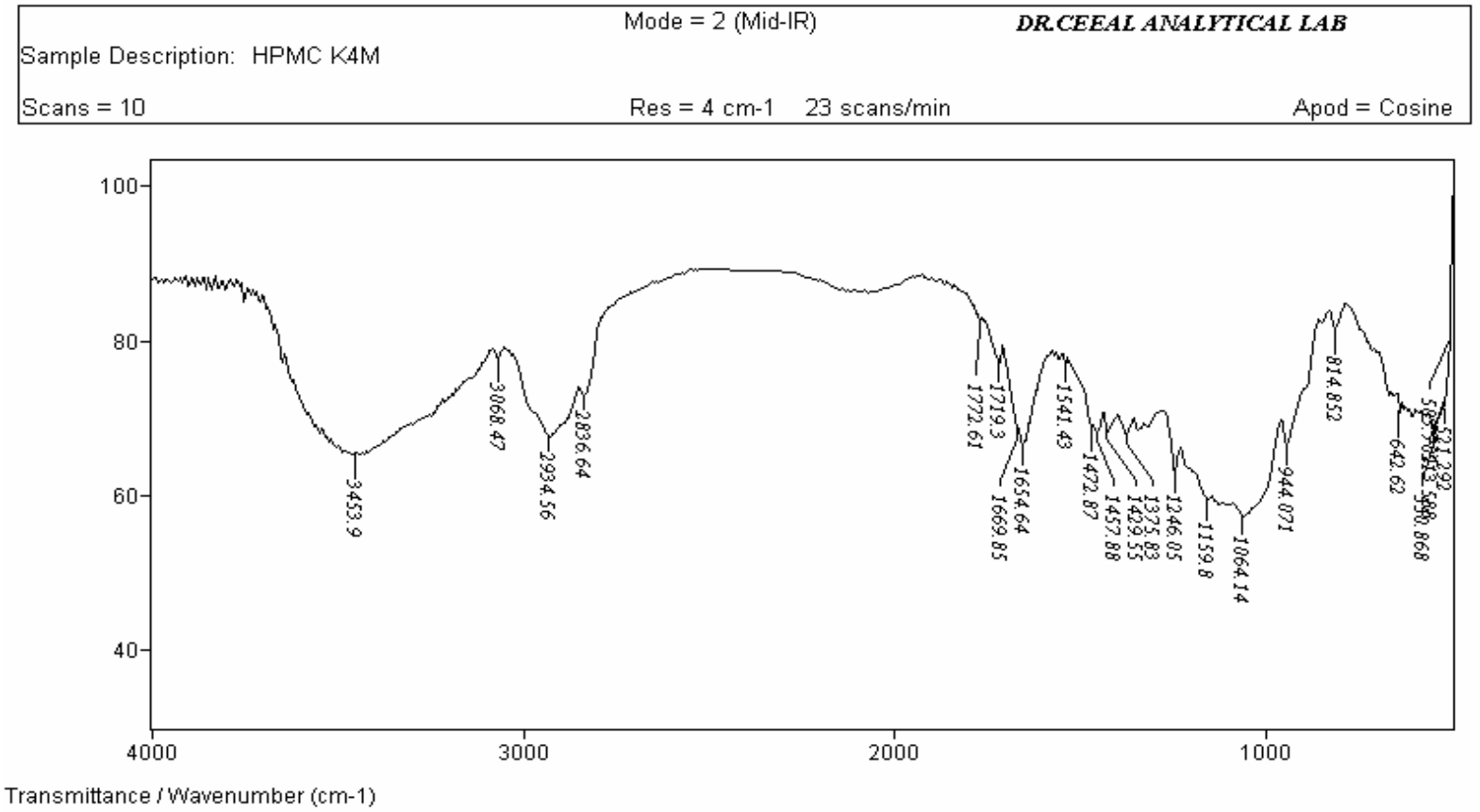


Fig 4.10: IR spectra of Drug and HPMC K4 M

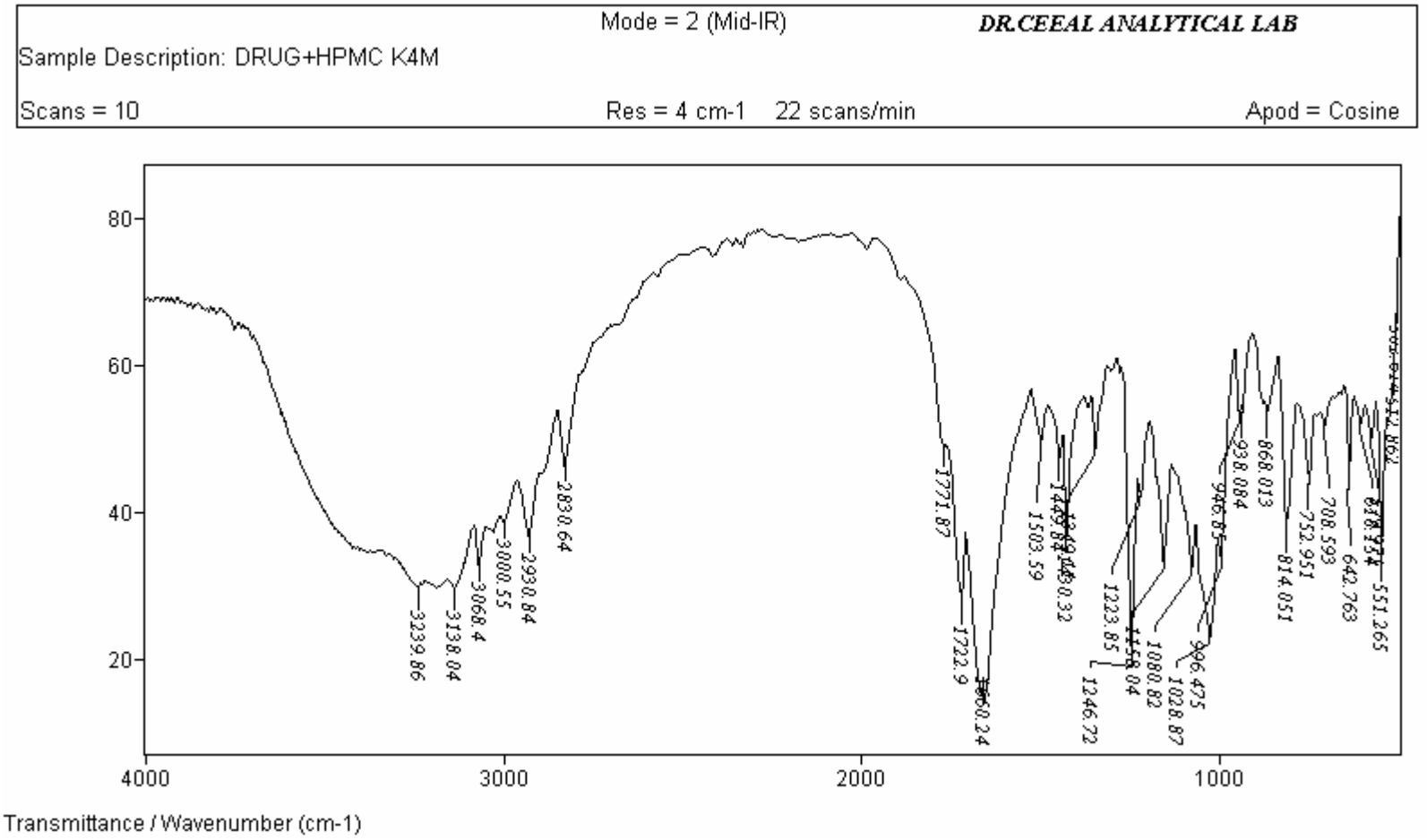


Fig 4.16: IR spectra of Drug, HPMC K100, Eudragit and β Cyclodextrin

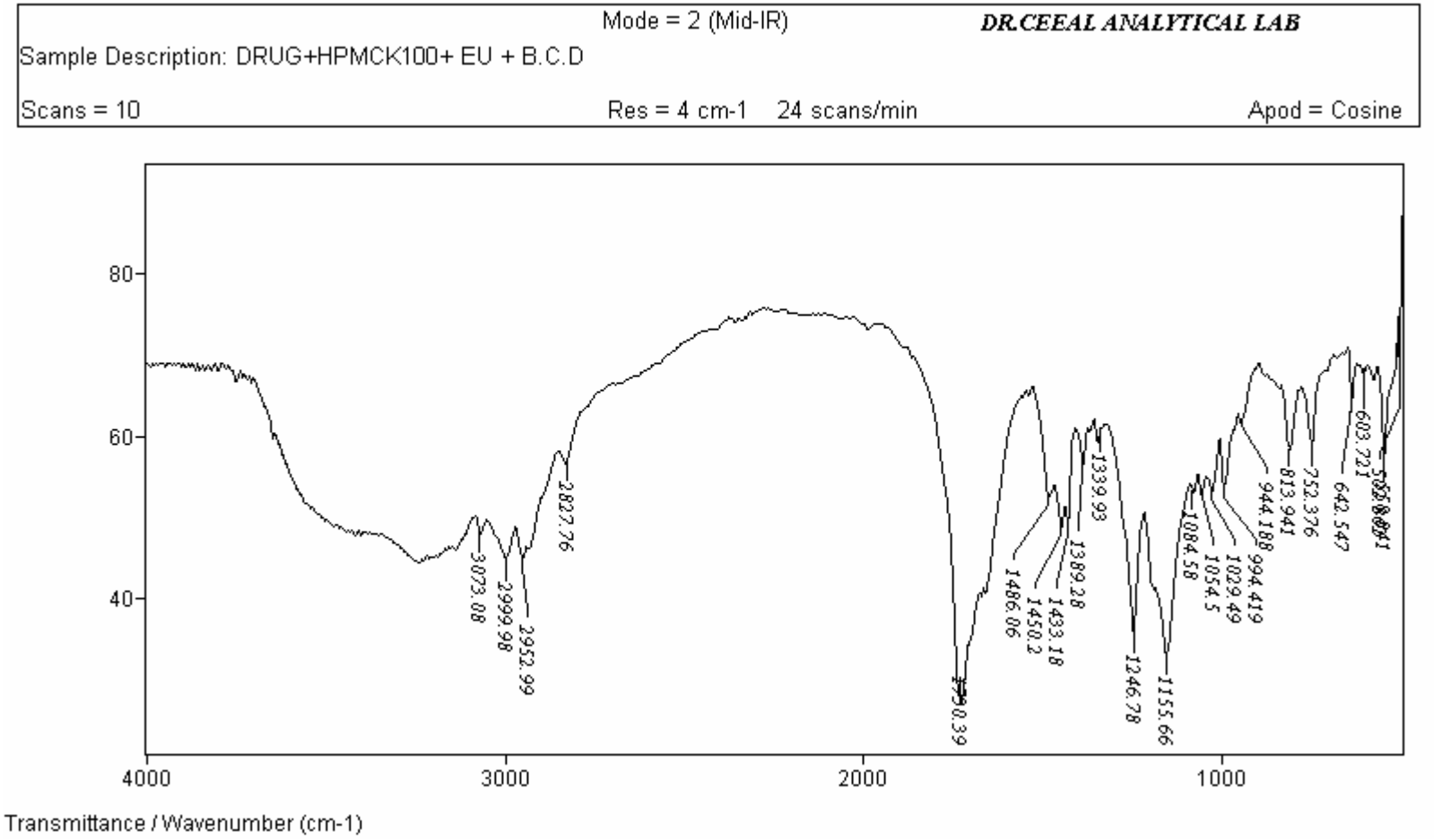
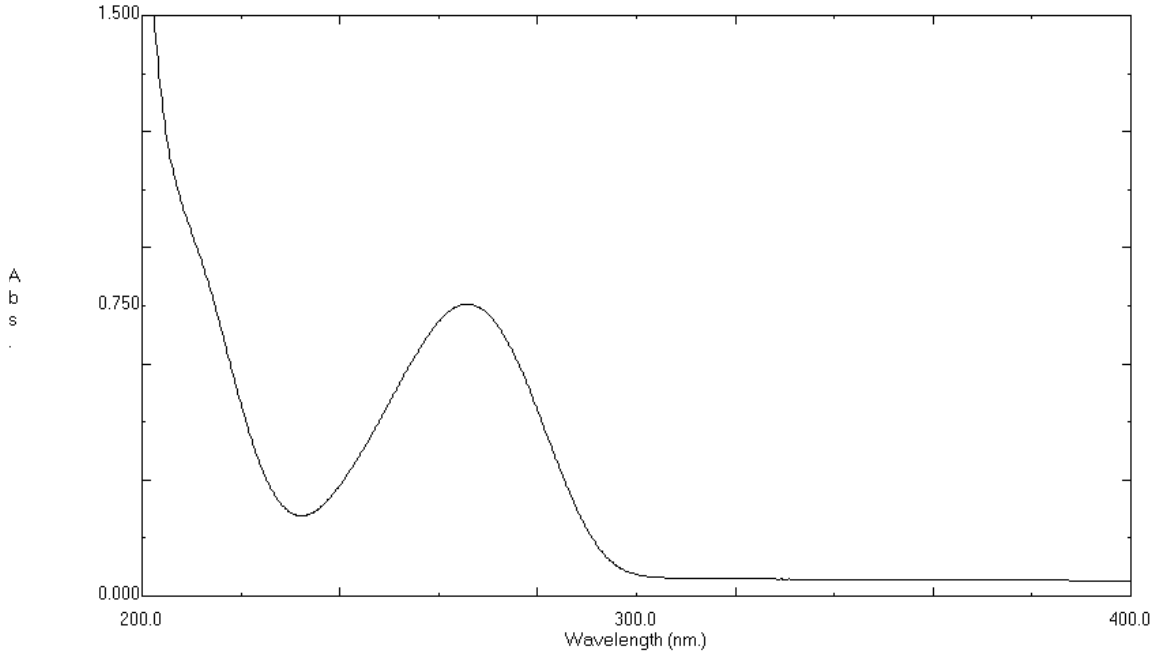


Fig 4.1: UV spectroscopy of 5 fluorouracil



Data: Original

Measuring Mode: Abs.
Scan Speed: Fast
Slit Width: 2.0
Sampling Interval: 0.2

No.	Wavelength (nm.)	Abs.
1	266.00	0.7540

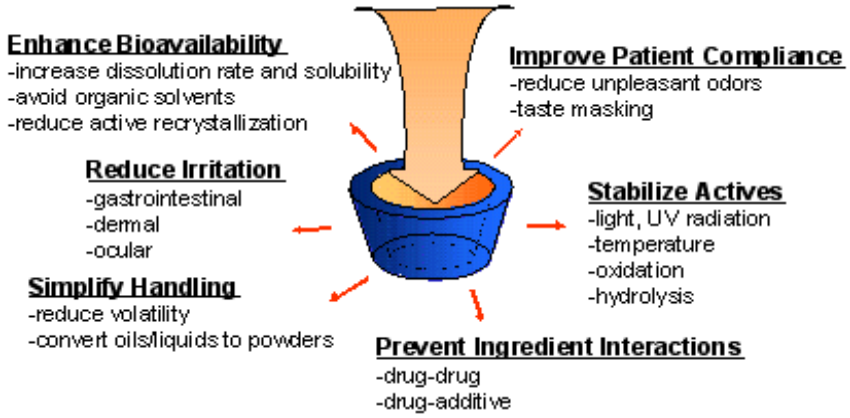
STUDIES ON COMPLEXATION OF 5 FLUOROURACIL WITH CYCLODEXTRIN

CYCLODEXTRINS FOR PHARMACEUTICAL APPLICATIONS

Cyclodextrins are bucket-shaped oligosaccharides produced from starch. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity[158]. The resulting inclusion complexes offer a number of potential advantages in pharmaceutical formulations (Figure 5.1).

Cyclodextrins increase the water solubility of poorly soluble drugs to improve their bioavailability. Light, thermal and oxidative stability of actives can be improved through the formation of cyclodextrin complexes. Cyclodextrins have also been used to reduce dermal, gastrointestinal or ocular irritation, mask unpleasant tastes or odors, prevent adverse drug-ingredient interactions and convert oils/liquids into powders to improve handling.

Figure 5.1. Multiple benefits exist for cyclodextrin complexes in pharmaceutical formulations.



Method of preparation, viz co-grinding, kneading, solid dispersion, solvent evaporation, co-precipitation, spray drying, or freeze drying can affect drug/CD

complexation. The effectiveness of a method depends on the nature of the drug and CD. In many cases, spray drying, and freeze drying were found to be most effective for drug complexation.

Table 5.1. Cyclodextrin characteristics

Cyclodextrin Type	α	β	γ
Number of Glucose Units	6	7	8
Appearance	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Molecular Weight	973	1135	1297
Bulk Density, g/cm^3	0.4 - 0.7	0.4 - 0.7	0.4 - 0.7
Content (dry basis)	>98%	>98%	>98%
Specific Rotation in Aqueous Solution$[\alpha]_{D,20}$	+147° to +152°	+160° to +164°	+174° to +180°
Water	<10%	<14%	<11%
Heavy Metals	<5 ppm	<5 ppm	<5 ppm
Residue on Ignition	<0.1%	<0.1%	<0.1%
Volatile Organics	<20 ppm	<5 ppm	<50 ppm
Micro-organisms	<1000/g	<1000/g	<1000/g

In addition, all these cyclodextrins exhibit good flow properties and handling characteristics[159] and are:

thermally stable (< 200 °C)

- very stable in alkaline solutions (pH < 14)
- stable in acidic solutions
- biocompatible

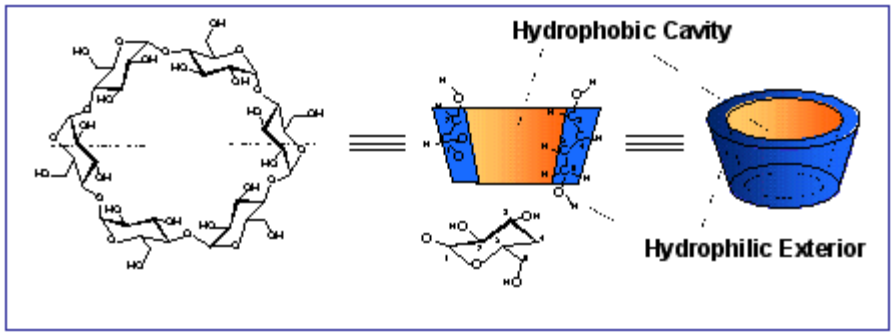
Cyclodextrin Complexes

The ability of a cyclodextrin to form an inclusion complex with a guest molecule is a function of two key factors. The first is steric and depends on the relative size of the cyclodextrin to the size of the guest molecule or certain key functional groups within the guest. If the guest is of wrong size, it will not fit properly into the cyclodextrin cavity. The second critical factor is the thermodynamic interactions between the different components of the system (cyclodextrin, guest, solvent). For a complex to form there must be a favorable net energetic driving force that pulls the guest into the cyclodextrin.

While the height of the cyclodextrin cavity is the same for all three types, the number of glucose units determines the internal diameter of the cavity and its volume .Based on these dimensions, α -cyclodextrin can typically complex low molecular weight molecules or compounds with aliphatic side chains, β -cyclodextrin will complex aromatics and heterocycles and γ -cyclodextrin can accommodate larger molecules such as macrocycles and steroids.

The most stable three dimensional structures of cyclodextrins is a toroid with the larger and smaller openings presenting hydroxyl groups to the external environment and mostly hydrophobic functionality lining the interior of the cavity (Figure 5.4). It is this unique configuration that gives cyclodextrins their interesting properties and creates the thermodynamic driving force needed to form host-guest complexes with apolar molecules and functional groups.

Figure 5.2. The conformation of the glucose units in the cyclodextrin



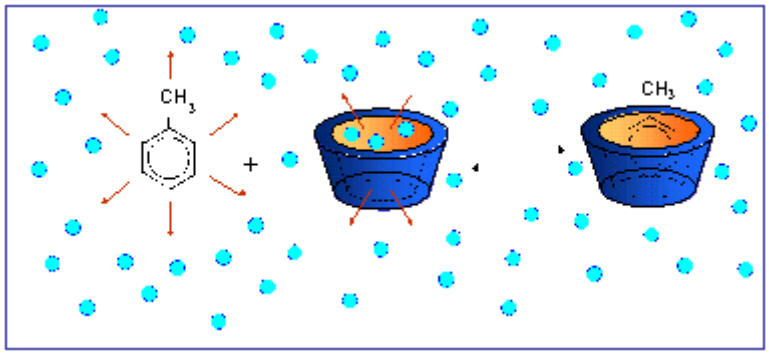
Glucose units in the cyclodextrin places the hydrophilic hydroxyl groups at the top and bottom of the three dimensional ring and the hydrophobic glycosidic groups on the interior.

Figure 5.3 provides a schematic representation of the equilibrium involved in forming an inclusion complex between cyclodextrin and toluene in the presence of a small amount of water. In general, there are four energetically favorable interactions that help shift the equilibrium to the right:

- the displacement of polar water molecules from the apolar cyclodextrin cavity
- the increased number of hydrogen bonds formed as the displaced water returns to the larger pool
- a reduction of the repulsive interactions between the hydrophobic guest and the aqueous environment
- an increase in the hydrophobic interactions as the guest inserts itself into the apolar cyclodextrin cavity

While this initial equilibrium to form the complex is very rapid[160] (often within minutes), the final equilibrium can take much longer to reach. Once inside the cyclodextrin cavity, the guest molecule makes conformational adjustments to take maximum advantage of the weak Van Der Waals forces that exist.

Figure 5.3. Forming an inclusion complex involves multiple interactions between active, solvent and cyclodextrin.



Complexes can be formed by a variety of techniques that depend on the properties of the drug, the equilibrium kinetics, the other formulation ingredients and processes and the final dosage form desired. However, each of these processes depends on a small amount of water to help drive the thermodynamics. Among the methods used are simple dry mixing, mixing in solutions and suspensions followed by a suitable separation, the preparation of pastes and several thermo-mechanical techniques.

Dissociation of the inclusion complex is a relatively rapid process usually driven by a large increase in the number of water molecules in the surrounding environment. The resulting concentration gradient shifts the equilibrium in Figure 5.3 to the left. In highly dilute and dynamic systems like the body, the guest has difficulty finding another cyclodextrin to reform the complex and is left free in solution.

Applications

As a result of their unique ability to form inclusion complexes[161], cyclodextrins provide a number of benefits in pharmaceutical formulations. Many of these applications have been well-studied and a significant amount of information exists in the technical literature. However, it is only recently that cyclodextrins have started to become commercially significant as production improvements have made them more economically available in large scale and formulators and regulatory agencies become more familiar with their properties.

Table 5.2. **Examples of CD-enhanced Solubility and Dissolution**

CD	Drug
β-CD	Nimesulide, Sulfomethiazole, Lorazepam, Ketoprofen, Griseofulvin, Praziquantel, Chlorthalidone, Etodolac, Piroxicam,, Itraconazole, Ibuprofen
α-CD	Praziquantel
γ-CD	Praziquantel, Omeprazole, Digoxin
HP-β-CD	Albendazole, DY–9760e, ETH–615, Levemopamil HCl, Sulfomethiazole, Ketoprofen,, Griseofulvin, Itraconazole, Carbamazepine Zolpidem, Phenytoin, Rutin
DM-β-CD	Naproxen, Camptothesin
SBE-β-CD	DY– 9760e, Danazol, Fluasterone, Spiranolactone
RM-β-CD	ETH–615, Tacrolimus
Randomly acetylated amorphous-β-CD (AC-β-CD)	Naproxen

CD Effects on Important Drug Properties in Formulation

Effect on Drug Solubility and Dissolution

CDs have been playing a very important role in formulation of poorly water-soluble drugs by improving apparent drug solubility and/or dissolution[162] through inclusion complexation or solid dispersion, by acting as hydrophilic carriers for drugs with inadequate molecular characteristics for complexation. It is also used as tablet dissolution enhancers for drugs with high dose, with which use of a drug/CD complex is difficult, eg, paracetamol. CD applications as solubilizing agents are summarized in Table 5.2.

Out of various commercially available CDs, methylated CDs with a relatively low molar substitution appear to be the most powerful solubilizers. Reduction of drug crystallinity on complexation or solid dispersion with CDs also contributes to the CD increased apparent drug solubility and dissolution rate. CDs, as a result of their ability to form in situ inclusion complexes in dissolution medium, can enhance drug dissolution even when there is no complexation in the solid state. CDs can also act as release enhancers, for example β-CD enhanced the release rate of poorly soluble naproxen and ketoprofen from inert acrylic resins and hydrophilic swellable (high-viscosity hydroxy propyl methyl cellulose [HPMC]) tableted matrices. β-CD also enhanced the release of theophylline from HPMC matrix by increasing the apparent solubility and dissolution rate of the drug[163,164].

Effect on Drug Bioavailability

CDs enhance the bioavailability[165] of insoluble drugs by increasing the drug solubility, dissolution, and/or drug permeability. CDs increase the permeability of insoluble, hydrophobic drugs by making the drug available at the surface of the biological barrier, eg, skin, mucosa, or the eye cornea, from where it partitions into the membrane without disrupting the lipid layers of the barrier. CDs increased the bioavailability of lipophilic itraconazole from both an oral solution and an intravenous formulation by improving the drug solubility and absorption.

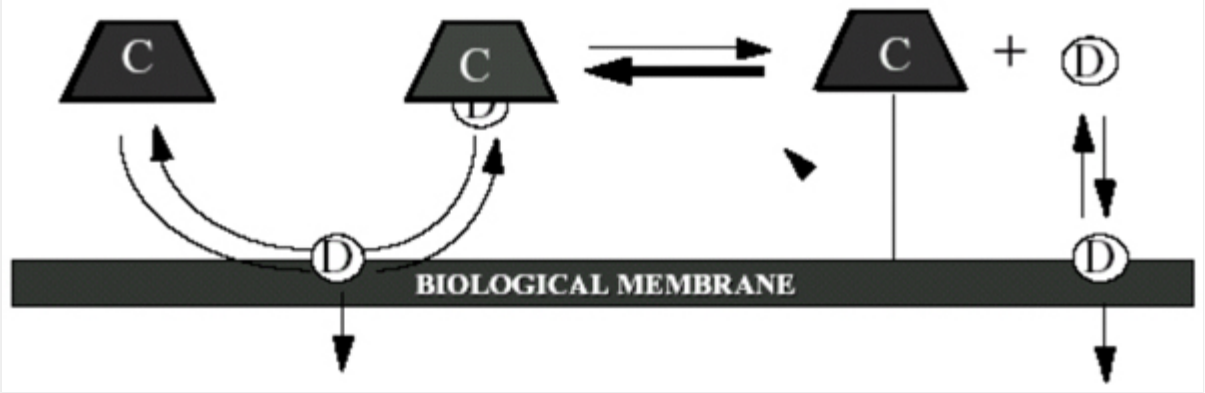


Figure 5.4. Mode of penetration enhancement by CDs.

CDs were reported to solubilize membrane components without entering into the membrane, and hence the perturbing effects of CDs can be mild and reversible. Labile drug stabilization by CDs and their ability to ameliorate drug irritation, and thus improve drug contact time at the absorption site in nasal, ocular, rectal, and transdermal delivery are some other important factors that contribute to the CD-improved bioavailability.

Effect on Drug Safety

CDs have been used to ameliorate the irritation caused by drugs. The increased drug efficacy and potency (ie, reduction of the dose required for optimum therapeutic activity), caused by CD-increased drug solubility, may reduce drug toxicity by making the drug effective at lower doses. β-CD enhanced the antiviral activity of ganciclovir on human cytomegalovirus clinical strains and the resultant increase in the drug potency reduced the drug toxicity. Further CD entrapment of drugs at the molecular level prevents their direct contact with biological membranes and thus reduces their side effects (by decreasing drug entry into the cells of nontargeted tissues) and local irritation with no drastic loss of therapeutic benefits. In a study with patients, piroxicam/β-CD inclusion complex showed better tolerance with lower incidence and severity of gastrointestinal side effects compared with the free drug.

Effect on Drug Stability

CDs can improve the stability[166] of several labile drugs against dehydration, hydrolysis, oxidation, and photodecomposition and thus increase the shelf life of drugs. It was reported that CD-induced enhancement of drug stability may be a result of inhibition of drug interaction with vehicles and/or inhibition of drug bioconversion at the absorption site. By providing a molecular shield, CD complexation encapsulates labile drug molecules at the molecular level and thus insulates them against various degradation processes. SBE-β-CD showed greater stability enhancement of many chemically unstable drugs than other CDs.

CD Applications in Drug Delivery

Oral Drug Delivery

Applications of CDs in oral drug delivery[167] include improvement of drug bioavailability due to increased drug solubility, improvement of rate and extent of dissolution, and/ or stability of the drug at the absorption site, eg, the gastrointestinal tract (GIT) or in formulation, reduction of drug-induced irritation, and taste masking (Table 5.3). CD complexation was found to decrease local drug irritation and also modify the time of drug release during GI transit. An itraconazole oral preparation containing 40% (wt/vol) of HP-β-CD (with reduced drug irritation) has been commercialized in the United States and Europe.

CDs enhance the mucosal drug permeability mainly by increasing the free drug availability at the absorptive surface. CD complexation can provide better and uniform absorption of low-soluble drugs with poor and erratic absorption[168] and also enhance the drug activity on oral administration. The relative safety, efficacy in terms of complexation, cost and acceptance in pharmacopeias are some important factors to be considered in selecting a CD for drug complexation. HP-β-CDs were shown to have a better oral safety profile than β-CD and other parent CDs, but only limited data are available on the oral safety of the methylated CDs. However, for oral administration all CDs can be considered practically nontoxic due to lack of CD absorption through GIT and, hence, the relative safety profile[169] of CDs is a concern of drug doses used in drug/CD complexes and the LD50 of CD.

Table 5.3. Applications of CDs in Oral Delivery

Effect	CD	Drug
↑ Bioavailability by ↑Solubility and dissolution rate	β-CD	Ketoprofen, Griseofulvin, Terfenadine
	HP-β-CD	Albendazole, Ketoprofen, Phenytoin, Gliclazide
	SBE–β-CD	Spiranolactone
	DM-β-CD	Tacrolimus
	M-β-CD	Albendazole
	ME-β-CD	Phenytoin
↑ Intensity or duration of therapeutic activity	β-CD	Terfenadine, Tolbutamide
	HP- β-CD	Tolbutamide, Amylobarbitone
↑ Permeability	HP-β-CD	Flutamide
↑Gastrointestinal stability	γ-CD	Digoxin
	HP-β-CD	Rutin
↑Sublingual bioavailability	HP-β-CD	Clomipramine, Testosterone

↑, increased effect

β-CD is the most cost-effective compound of all CDs, whereas HP-β- and SBE-β-CDs are more expensive. Monograph of β-CD is already incorporated in various pharmacopeias and national formularies (NF). Hence, β-CD can be considered optimum for oral use when it is effective for drug complexation and modified CDs like HP-, SBE-β-, and DM-β-CDs may be used when they are more effective and when their peculiar property is required in formulation, eg, SBE-β-CD, owing to its osmotic property, was used in the preparation of osmotic pump tablets.

Parenteral Drug Delivery

CD derivatives such as amorphous HP-β- and SBE-β-CDs have been widely investigated for parenteral use[170] on account of their high aqueous solubility and minimal toxicity. An itraconazole parenteral injection containing HP-β-CD (40% wt/vol) has been commercialized in the United States and Europe.

Ocular Delivery

Applications of CDs in aqueous eye drop preparations include solubilization and chemical stabilization of drugs, reduction of ocular drug irritation, and enhancement of ocular drug permeability.

Nasal Drug Delivery

CDs are effective excipients in nasal drug delivery. CDs improve nasal drug absorption either by increasing aqueous drug solubility and/or by enhancing nasal drug permeability. The safety and nontoxicity of CDs in nasal drug formulations have been demonstrated by the clinical data with CDs showing no adverse effects.

Rectal Drug Delivery

Applications of CDs in rectal delivery[171] include enhancing drug absorption from a suppository base either by enhancing drug release from the base or by increasing drug mucosal permeability, increasing drug stability in the base or at the absorption site, providing

sustained drug release, and alleviating drug-induced irritation.

Controlled Drug Delivery

CDs, due to their ability either to complex drugs or to act as functional carrier materials in pharmaceutical formulations, can serve as potential candidates for efficient and precise delivery of required amounts of drugs to targeted site for a necessary period of time. Hydrophilic and hydrophobic CD derivatives are used in immediate and prolonged release type formulations, respectively.

CD was also reported to have bioadhesive effects on gastrointestinal mucosa. CDs can also be used along with other carrier materials to optimize drug release rate. Improved nifedipine bioavailability with reduced first pass metabolism was observed from a modified oral dosage form containing a fast release portion of the drug with HP–β-CD and HCO-60, a nonionic surfactant (ie, amorphous drug form obtained by spray drying with the CD and surfactant) and a slow release portion with hydroxy propyl celluloses (HPCs) of different viscosity grades.

Colon-Specific Drug Delivery

CDs are barely hydrolyzed and only slightly absorbed in the stomach and small intestine but are absorbed in the large intestine after fermentation into small saccharides by colonic microbial flora. The peculiar hydrolyzing property of CDs makes them useful for colon drug targeting.

Peptide and Protein Delivery

Various problems associated in practical use of therapeutic peptides and proteins are their chemical and enzymatic instability, poor absorption through biological membranes, rapid plasma clearance, peculiar dose response curves, and immunogenicity. β-CD improved insulin loading of alginate microspheres prepared by an emulsion-based process.

Gene and Oligonucleotide Delivery

The toxicity and immunogenicity associated with viral vectors led to the development of

nonviral vectors for gene delivery. Besides the plasmid or virus-based vector systems, "naked" nucleotide derivatives have also been investigated for possible use as therapeutic agents through several routes of administration.

Dermal and Transdermal Delivery

CDs have been used to optimize local and systemic dermal drug delivery[172]. Applications of CDs in transdermal drug delivery include enhancement of drug release and/or permeation, drug stabilization in formulation or at absorptive site, alleviation of drug-induced local irritation, sustaining of drug release from vehicle, and alteration of drug bioconversion in the viable skin.

Brain Drug Delivery or Brain Targetting

The concept of Bodor's chemical delivery system (CDS) (ie, covalent coupling of drugs to 1-methyl-1, 4-dihydronicotinic acid through an enzymatically labile linkage, which increases drug lipophilicity) was applied for targeting drugs such as steroids, antitumor agents, and calcium channel antagonists to brain. Use of CDs in the formulation of CDS can be demonstrated by the significantly improved solubility, stability, and pharmacologic activity of CDS of thyrotropin-releasing hormone analogs on complexation with HP-β-CD.

CD Applications in the Design of Some Novel Delivery Systems

Liposomes

In drug delivery, the concept of entrapping CD-drug complexes into liposomes[173] combines the advantages of both CDs (such as increasing the solubility of drugs) and liposomes (such as targeting of drugs) into a single system and thus circumvents the problems associated with each system.

Microspheres

In the presence of a high percentage of highly soluble hydrophilic excipients, complexation

may not improve the drug dissolution rate from microspheres. Nifedipine release from chitosan microspheres was slowed down on complexation with HP-β-CD in spite of the improved drug-loading efficiency. Study of in vivo release behavior (over 24 hours) of β-CD from β-CD/poly (acrylic acid) (PAA) microspheres, prepared by a water/oil solvent evaporation technique, indicated a high encapsulating efficiency (>90%) with potential covalent binding of the CD. The amount of CD linked in microspheres was in the order β- > γ- > α-CD and the dimensions of the microspheres with γ-CD were much higher than those with α- or β-CDs.

Microcapsules

It was suggested that crosslinked β-CD microcapsules[174], because of their ability to retard the release of water-soluble drugs through semi permeable membranes, can act as release modulators to provide efficiently controlled release of drugs.

Nanoparticles

Nanoparticles are stable systems suitable to provide targeted drug delivery and to enhance the efficacy and bioavailability of poorly soluble drugs. However, the safety and efficacy of nanoparticles are limited by their very low drug loading and limited entrapment efficiency (with classical water emulsion polymerization procedures) that may lead to excessive administration of polymeric material. Two applications of CDs have been found very promising in the design of nanoparticles: one is increasing the loading capacity of nanoparticles and the other is spontaneous formation of either nanocapsules or nanospheres by nanoprecipitation of amphiphilic CDs diesters.

CD Use as Excipients in Drug Formulation

As excipients[175], CDs have been finding different applications in the formulation and processing of drugs. β-CD, due to its excellent compactability (varied with source) and minimal lubrication requirements, showed considerable promise as a filler binder in tablet manufacturing but its fluidity was insufficient for routine direct compression. β-CD was also

found to be useful as a solubility enhancer in tablets. Complexation can cause subtle changes in the tabletting properties of drugs[176] or CDs that can substantially affect the stability and tabletting performance of tablet formulations containing drug/CD complexes.

CDs, as a result of their complexation ability and other versatile characteristics, are continuing to have different applications in different areas of drug delivery and pharmaceutical industry. However, it is necessary to find out any possible interaction between these agents and other formulation additives because the interaction can adversely affect the performance of both. It is also important to have knowledge of different factors that can influence complex formation in order to prepare economically drug/CD complexes with desirable properties[177]. Since CDs continue to find several novel applications in drug delivery, we may expect these polymers to solve many problems associated with the delivery of different novel drugs through different delivery routes.

MATERIALS AND METHODS

MATERIALS

5-fluorouracil, β-cyclodextrin was purchased from Sigma Chemical Co (St Louis, MO); both were used as received with no further purification. All other reagents and chemicals were of analytical grade.

METHODS

Phase-Solubility Study

The phase solubility technique permits the evaluation of the affinity between β-cyclodextrin and 5-fluorouracil in water. Phase solubility studies were performed according to the method reported by Higuchi and Connors.[178] 5-fluorouracil, in amounts that exceeded its solubility, was taken into vials to which were added 15 ml of distilled water (pH 6.8) containing various concentrations of β-cyclodextrin (3-15 mM). These flasks were sealed and shaken at 20°C for 5 days. This amount of time is considered sufficient to reach equilibrium. Subsequently, the aliquots were withdrawn, using a syringe, at 1hour intervals, and samples were filtered immediately through a 0.45-μ nylon disc filter and appropriately diluted. A portion of the sample was analyzed by UV spectrophotometer at 266nm against blanks prepared in the same concentration of β-cyclodextrin in water so as to cancel any absorbance that may be exhibited by the β-cyclodextrins. Shaking was continued until 3 consecutive estimations were equivalent. The solubility experiments were conducted in triplicate.

Preparation of Solid Complexes

The preparation of solid complexes of 5-fluorouracil and β-cyclodextrin were performed by different techniques[179] like physical mixture, kneading method, freeze drying and co evaporation, which are described below in detail. The molar ratio was kept as 1:1 and 1:2 (5-fluorouracil: β-cyclodextrin). Eight formulations were prepared.

Physical Mixture

Physical mixtures were prepared by homogeneous blending of previously sieved and weighed 5-fluorouracil and β-cyclodextrin in a mortar.

Kneading Method

β-cyclodextrin and distilled water were mixed together in a mortar so as to obtain a homogeneous paste. 5-fluorouracil was then added slowly; while grinding. The mixture was then ground for 1 hour. During this process, an appropriate quantity of water was added to the mixture in order to maintain a suitable consistency. The paste was dried in oven at 40°C for 24 hours. The dried complex was pulverized into a fine powder.

Freeze-Drying Method

The required quantity of 5-fluorouracil was added to an aqueous solution of β-cyclodextrin while mixing with a magnetic stirrer. After 24 hours of agitation, the resulting solution was frozen by keeping it in a repository at 60°C and was lyophilized in a freeze-dryer for 24 hours.

Co Evaporation Method

After dissolution of β-cyclodextrin in water, the molar proportion of 5-fluorouracil was added. This suspension was further kept under stirring for 24 hours. The obtained clear solution was evaporated under vacuum at a temperature of 45°C and 100 rpm in a rotary evaporator. The solid residue was further dried completely at 40°C for 48 hours.

EVALUATION

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were recorded for complexes prepared, in potassium bromide (KBr) disks using a FTIR-5300 spectrophotometer. Samples were prepared in KBr disks by means of a hydrostatic press. The scanning range was 400 to 4000 cm^{-1} and the resolution was 4 $cm^{-1.}$

Differential Scanning Calorimetry

The DSC measurements[179] were performed using a DSC 200 PC Series (Perkin Elmer) DSC module controlled by STAR^{e} software (Mettler -Toledo GmbH, Switzerland). All accurately weighed samples (1 mg of 5-fluorouracil or its equivalent) were placed in sealed aluminum pans, before heating under nitrogen flow (20 ml/min) at a scanning rate of 10°C min^{-1}, over the temperature range of 30°C to 220°C. An empty aluminum pan was used as reference.

Dissolution Rate Studies

The dissolution behaviors of the 5-fluorouracil-β-cyclodextrin complexes[180,181] were compared with those of pure 5-fluorouracil. The dissolution rate studies were performed according to the United States Pharmacopoeia (USP) XXII rotating basket method. The samples, corresponding to 100 mg of 5-fluorouracil, were placed into hard gelatin capsules. The dissolution medium was 900 ml of SGF (simulated gastric fluid) without enzymes. The stirring speed was 100 rpm, and the temperature was maintained at 37°C ± 1°C. The samples (5 ml) were withdrawn at various time intervals using a pipette, filtered through 0.45 μ nylon disc filter, and analyzed by UV spectrophotometer at 266 nm.

Molecular-Modeling Studies

Molecular mechanics and dynamics calculations were performed with the Insight II/Discover program (Molecular Simulations Inc, San Diego, CA) using consistent valence force field (CVFF) on an SGI Octane platform (Silicon Graphics Inc, Mountain

View, CA). The structure of β-cyclodextrin was taken from the Cambridge Structural Database (Reference code PIJGIY)[182] The β-cyclodextrin monomer was constructed according to the experimental procedure of Bonnet et al[183]. The 5 FU molecules were drawn using the builder program of Insight II. The structure was then energy minimized with several algorithms (at first, steepest descent, followed by conjugate gradient to refine the structure) until the derivative was less than 0.01 kcal mol. The Electrostatic Potential (ESP) changes were extracted using AM_1 calculations.

To fit the 5 FU into the cavity of CD, Monte Carlo docking simulations were performed with the refined structures. Several initial configurations were tried. Each cycle began with a random change of up to 5 degrees of freedom among them. If the energy of the resulting host-guest system was within 1000 kcal/mol from the previous accepted structure, the system was subjected to 100 interactions of conjugate gradient energy minimization. The nonbonded interactions were calculated by cell-multipole method, and the dielectric constant was set to 1.

One of the low-energy structures of the docking simulations of each host-guest complex was subjected to molecular-dynamics (MD) simulation. MD simulations were performed in vacuo. The MD calculations were done using the velocity verlet algorithm at constant volume with the cell-multipole method[184] for the calculation of nonbonded interactions. The initial atomic velocities were assigned from a Guassian distribution corresponding to a temperature of 298 K. The system was equilibrated for 100 ps and the production run was done for 250 ps with a time step of 1 fs. Intermediate structures were saved every 100 fs for analysis. Solvent and counter-ion effects were simulated using a distance-dependent dielectric constant[185] with E = crij, where c = 3.5 during Molecular-Mechanics (MM) stages and 1 during MD stage.

RESULTS AND DISCUSSION

Phase-Solubility Study

The phase solubility diagram for the complex formation between 5-fluorouracil and β-cyclodextrin is presented in Figure 1 and Table 1 respectively. This plot shows that the aqueous solubility of the drug increases linearly as a function of β-cyclodextrin concentration. It is clearly observed that the solubility diagram of 5-fluorouracil in the presence of β-cyclodextrin can be classified as the B type curve. The host guest correlation with slope of less than 1 (0.19363) suggested the formation of a 1:1 (5-fluorouracil-β-cyclodextrin) complex with respect to β-cyclodextrin concentrations. Generally B type phase solubility behavior is typical for natural β-cyclodextrin, since the drug / CD complex is more soluble than the free drug itself but the solubility limit of the drug/CD complex is reached within the concentration range of the cyclodextrin.

FTIR studies

IR spectra of fluorouracil as such and in the complexes formed by various methods were identical. Principal IR absorption peaks of 5FU at 3124 cm^{-1} (NH Stretch), 1716 cm^{-1} and 1657 cm^{-1} (C=O Stretch), 1245 cm^{-1} (CH in Plane deformation), 813 cm^{-1} (CH out of plane deformation) were all observed in the spectra of 5 FU as well as its complexes. These spectral observations thus indicated that no interaction between 5 FU and β-cyclodextrin were seen in the complexation. (Fig 2a – 2f)

Differential Scanning Calorimetry

The DSC thermograms for the 5-fluorouracil and the corresponding β-cyclodextrin complexes assayed are represented in Figure 3a – 3f. As shown in the figure, 5-fluorouracil exhibits a characteristic endothermic fusion peak at 292.46°C; hence no polymorphs of 5-fluorouracil could be found. Furthermore, β-cyclodextrin shows a broad endothermic effect at 118.34°C. The DSC thermograms for the 5-fluorouracil-β-cyclodextrin systems show the persistence of the endothermic peak of 5-fluorouracil for the physical mixture and the kneaded product. For the freeze-dried and evaporated system, this peak is very small; this result can be explained on the basis of a major

interaction between the drug and cyclodextrin. Furthermore, the characteristic endothermic effect of β-cyclodextrin is slightly shifted to higher temperatures for the freeze-dried and evaporated systems, indicating that 5-fluorouracil has complexed with β-cyclodextrin.

Dissolution Rate Studies

The dissolution profiles of 5-fluorouracil alone and the 5-fluorouracil-β-cyclodextrin complexes are reported in Table 2 and 3 and Figure 4 and 5. The release rate profiles were drawn as the percentage of drug dissolved vs. time. According to these results, the inclusion complexes released up to 82% of the drug in 15 minutes, and up to 86% after 40 minutes; whereas 5-fluorouracil pure drug exhibited the release of ~22% after 20 minutes and not more than 36% after 60 to 90 minutes. These quantities contrast with the markedly 3-fold increase in the release of freeze-dried product.

It is also evident that the freeze-dried, evaporated, and kneaded systems exhibit higher dissolution rates than the physical mixture and the pure drug (Table 1). The extent of the enhancement of the dissolution rate was found to be dependent on the preparation method, since the freeze-dried and evaporated products exhibited the highest dissolution rates. The dissolution rate increase reached for the physical and kneaded mixtures is only due to the wetting effect of the β-cyclodextrin. In fact, this effect is more evident for the kneaded product, where the mixing process between the 2 components is more intensive. The effect of complexation with β-cyclodextrin on the solubility of 5-fluorouracil can be explained in terms of the reduction in the crystallinity of the drug caused by the freeze-drying process and the inclusion into the hydro-phobic cavity of the β-cyclodextrin.[179-181]

The complexes prepared by kneading technique offer a dissolution rate of approximately 65% in 60-minutes, which may be of particular interest for industrial scale preparations because of the low cost and the simple process, which involves less energy, time, and equipment.

Molecular-Modeling Studies

Molecular-Modeling Studies show the structures obtained by molecular modeling according to the methods described in the experimental section. The Monte Carlo (MC)

simulations showed a general tendency of inclusion complex formation and lowering interaction energy. The interaction energy was defined as the difference between the sum of the energy of individual host and guest molecule and the energy of the inclusion complex. The calculated energy value is -45.4 kcal/mol for structure 1, when 5 FU is docked through the head region of the β-cyclodextrin (ie, through the narrow rim [primary hydroxyl groups]). The energy value is -48.2 kcal/mol when 5 FU is introduced through the tail region of the β-cyclodextrin (ie, by the wider rim [secondary hydroxyl groups]).

The low energy confirmation of the β-cyclodextrin monomer-5 FU complex was found at -61.68 kcal/mol, indicating that the inclusion complex formation of the β-cyclodextrin monomer with 5 FU was energetically more favorable. In the β-cyclodextrin monomer, the guest molecule was fully embedded in the cavity

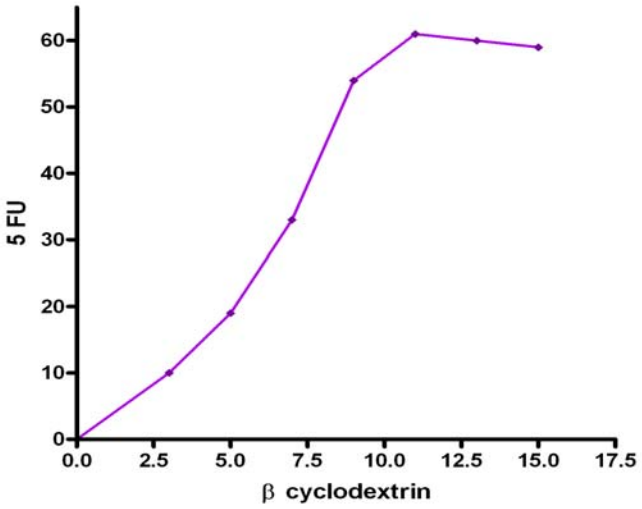
The general features of the MD trajectories were very similar to those from MC docking simulations. It showed overlap of snapshots from MD trajectories of 10 minimum energy structures. The calculations converge well with maximum violation of 0.42 A° and the Root Mean Square Deviation (RMSD) backbone of 0.95 A° for β-cyclodextrin and 0.87 A° for β-cyclodextrin monomer. It can be seen from the Figure 5B, that β-cyclodextrin monomer bound 5 FU tightly. The interaction energy for the lowest energy structure showed good agreement with the MC docking simulations. The interaction energies were -57.9 kcal/mol for β-cyclodextrin monomer, -36.5 kcal/mol for structure 1 and -37.2 kcal/mol for structure 2. These results indicate the relative energetic stability of the β-cyclodextrin-5 FU as in the case of MC docking simulations. A possible molecular arrangement for the inclusion compound is that the molecule 5 FU is buried in the cavity of the β-cyclodextrin monomer. It is being held in position due to the formation of hydrogen bonds between the hydroxyl groups of the β-cyclodextrin and the fluorine of the 5 FU. The contribution due to the electrostatic interactions is very small (ie, -0.45 kcal/mol. From the results it is clearly evident that a molar ratio of 1:1 (monomer) is suitable for β-cyclodextrin complexation of 5 fluorouracil.

Results obtained by different characterization techniques clearly indicate that the freeze-drying method in the ratio of 1:1 leads to formation of solid state complexes between 5-fluorouracil and β-cyclodextrin.

Table 1: Solubility study of the drug in complexes

RATIO	METHOD	SOLUBILITY mg/ml
Plain drug	-	12.1
1:2	Physical mixing	14.9
	Freeze drying	16.0
	Kneading	15.4
	Co-evaporation	13.35
1:1	Physical mixing	22.5
	Freeze drying	35.1
	Kneading	30.15
	Co-evaporation	17.24

Fig 1 Phase solubility study of the drug with β-cyclodextrin



FTIR STUDIES OF COMPLEXES

Fig 2a. IR Spectra of 5 Fluorouracil

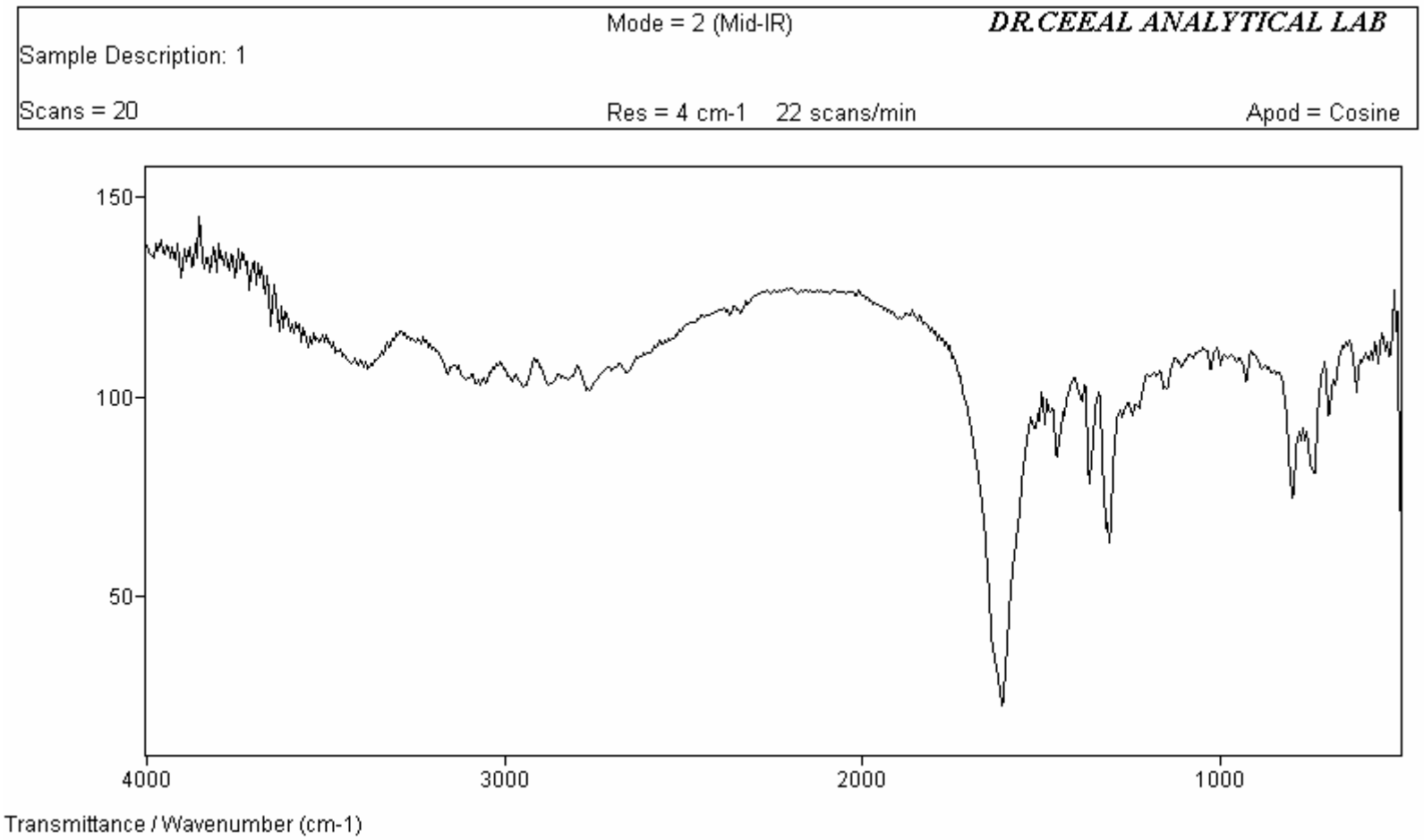


Fig 2b: IR Spectra of β Cyclodextrin

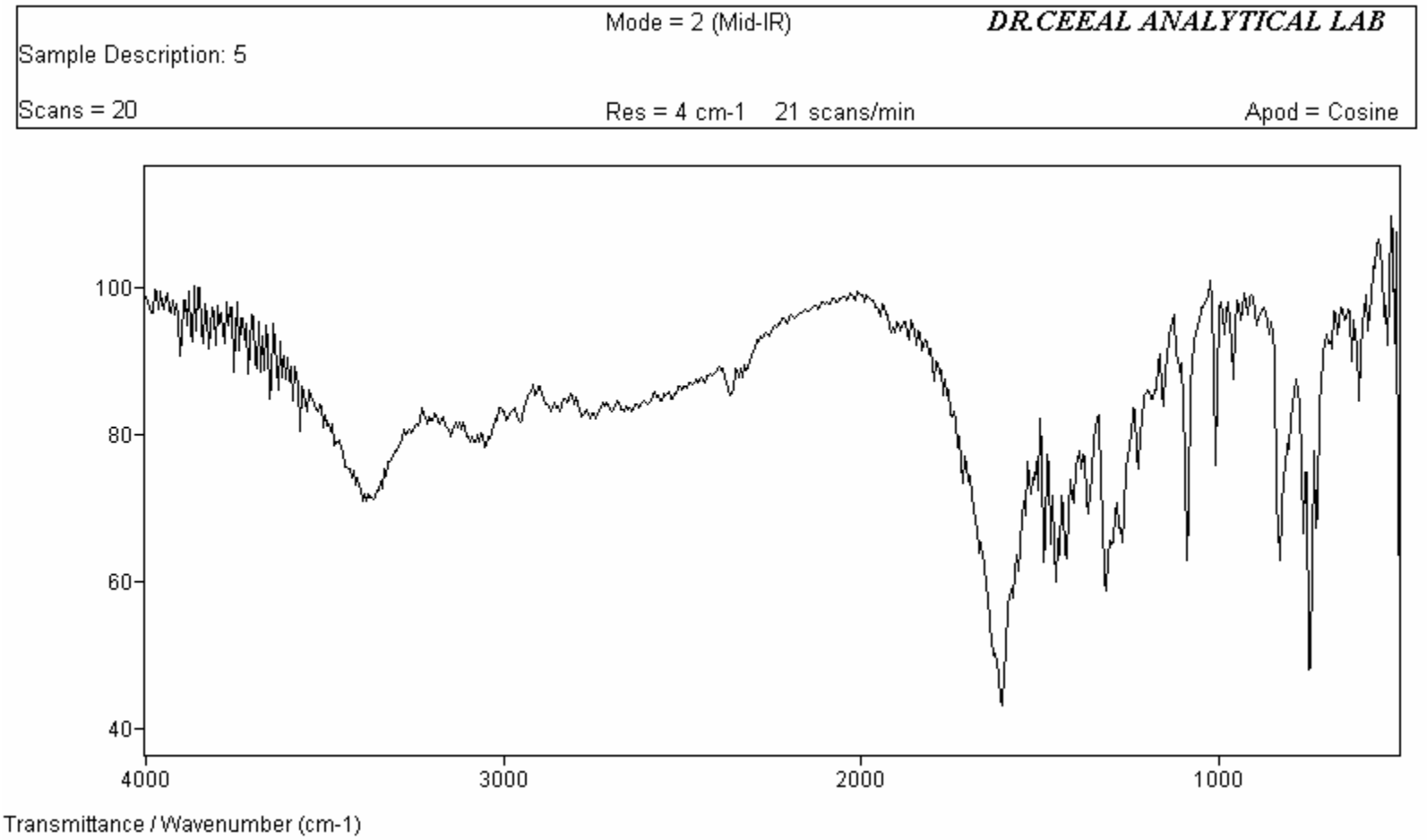


Fig 2c: IR Spectra of complex prepared by Physical Mixture method

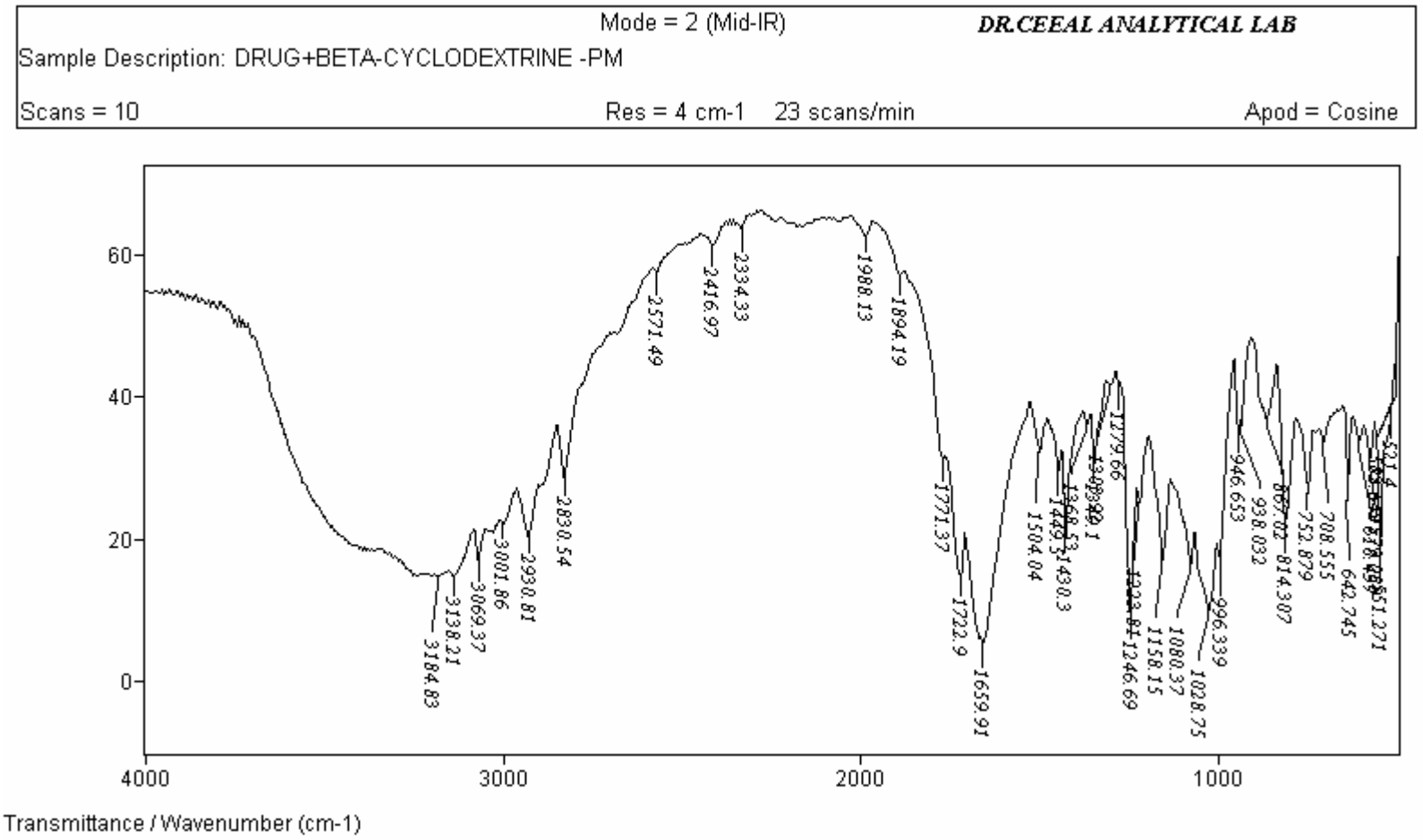
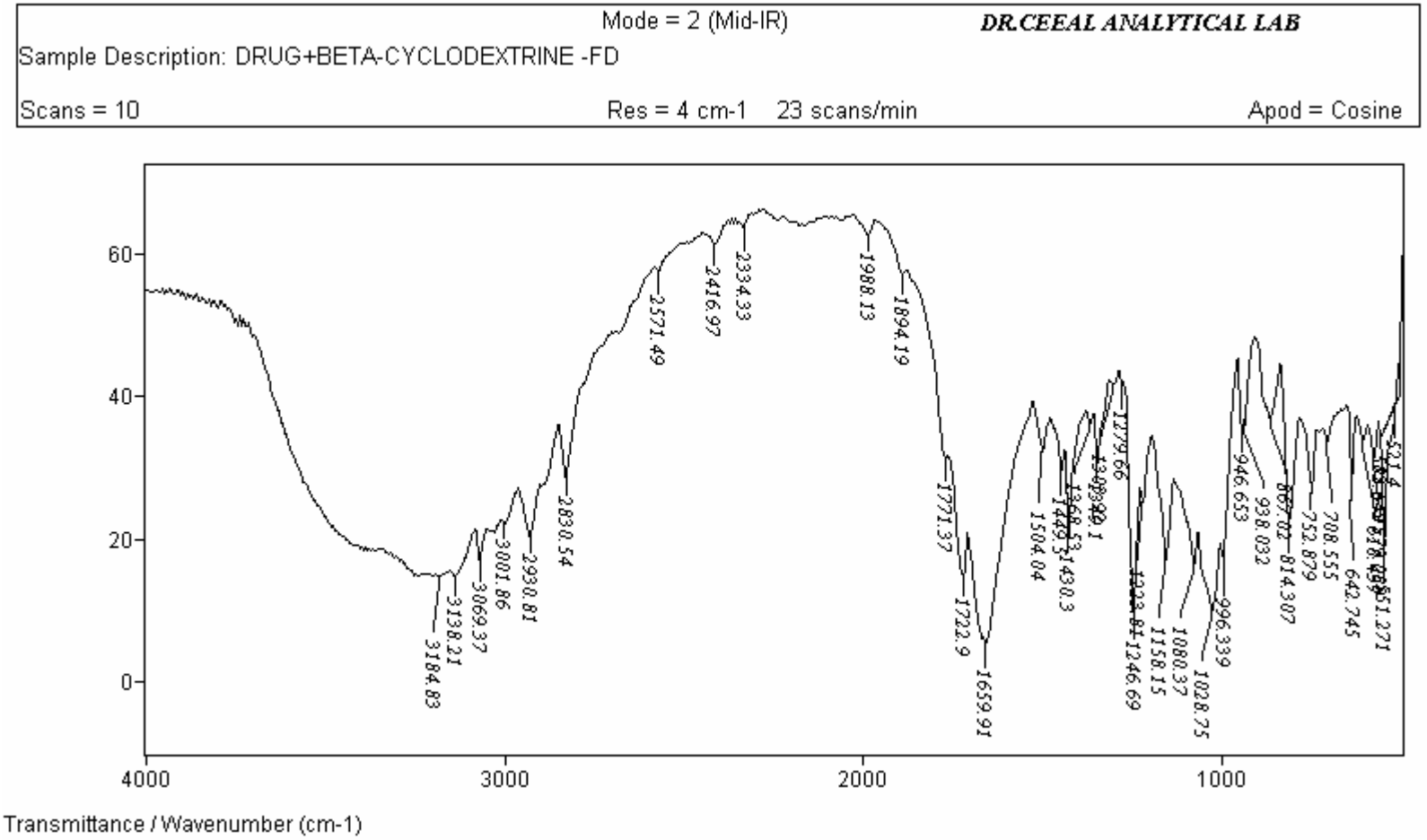


Fig 2d: IR Spectra of complex prepared by freeze drying method



Transmittance / Wavenumber (cm-1)

Fig 2e:IR Spectra of complex prepared by co evaporation method

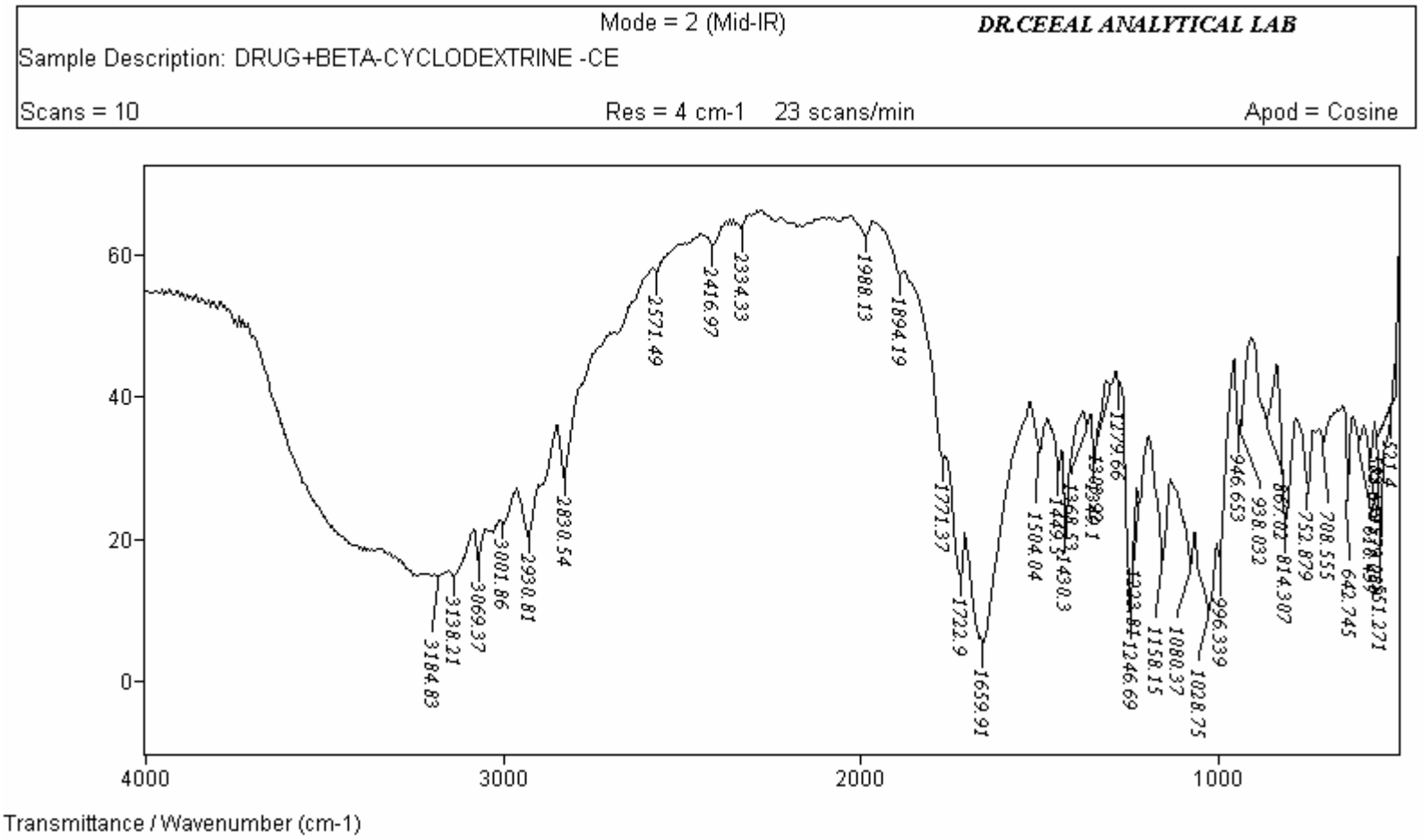


Fig 2f: IR Spectra of complex prepared by kneading method

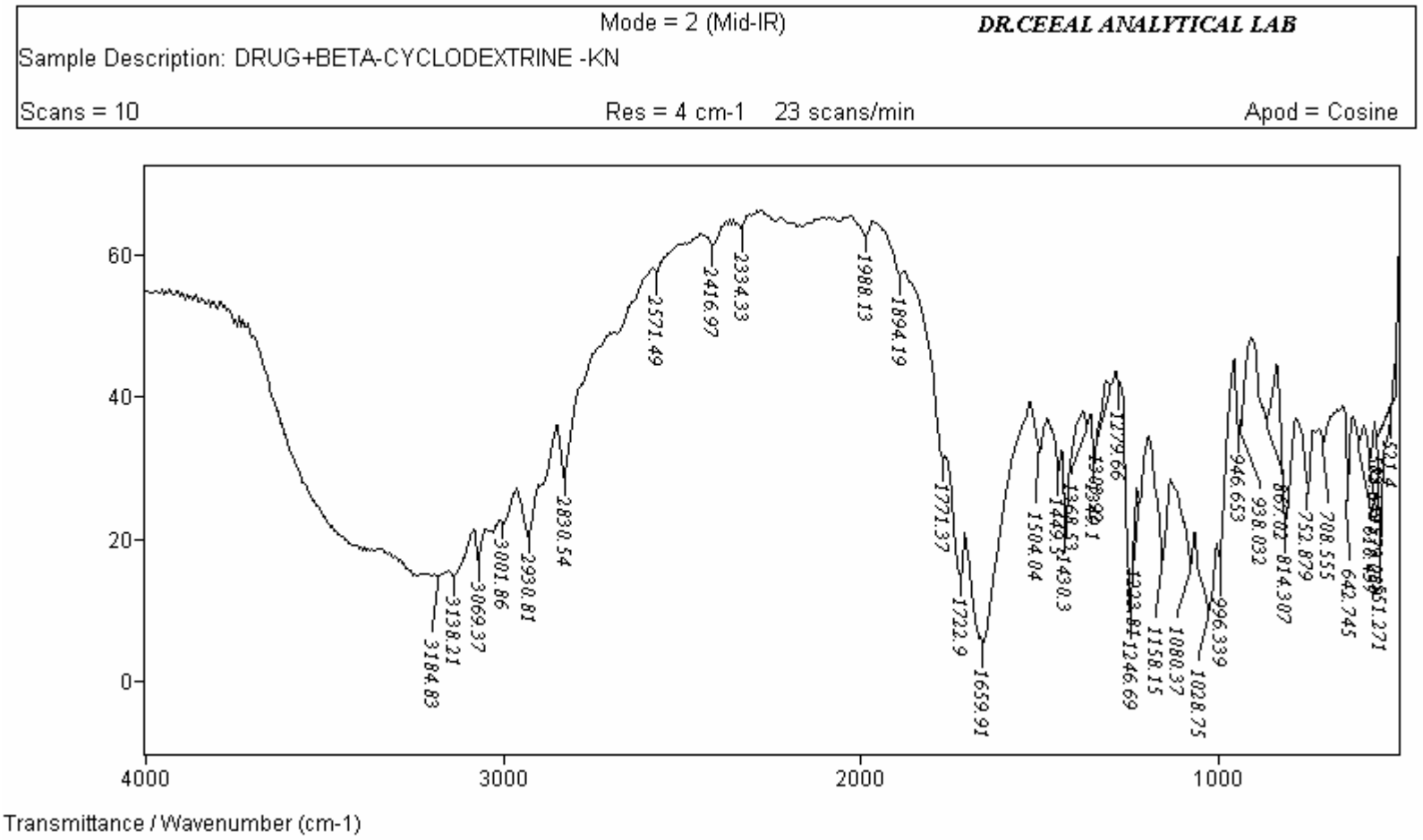


Table 2: Dissolution study of 5 fluorouracil and β-cyclodextrin complexes (1:1)

TIME	5 FU	PM	KM	CE	FD
0	0	0	0	0	0
10	20.32	39.74	52.49	71.29	79.62
20	22.18	40.56	55.18	72.38	82.02
30	22.94	41.97	56.36	74.88	85.24
40	24.43	43.12	59.74	75.73	86.67
50	27.82	46.85	61.69	77.16	89.85
60	30.55	47.22	65.09	79.82	91.12
75	32.79	51.66	67.23	80.21	91.93
90	36.18	53.14	68.35	81.13	92.46

5 FU – 5-fluorouracil, PM - physical mixture, KM - Kneading Method,

EM – Evaporation Method, FD – Freeze Drying

Table 3: Dissolution study of 5 fluorouracil and β-cyclodextrin complexes (1:2)

Time (min)	5 FU	PM	KM	CE	FD
0	0	0	0	0	0
10	20.32	27.41	39.7	26.25	42.85
20	22.18	37.7	41.3	36.14	51.45
30	22.94	30.0	55.6	39.04	63.56
40	24.43	32.9	56.9	40.04	64.36
50	27.82	41.3	67.3	42.96	69.1
60	30.55	41.9	68.08	44.20	70.8
75	32.79	48.3	70.23	49.5	72.9
90	36.18	55.8	71.1	56.3	74.3

5 FU – 5-fluorouracil, PM - physical mixtures, KM - Kneading Method,

CE – Evaporation Method, FD – Freeze Drying

Fig 4. Dissolution profile of 5-fluorouracil and its complexes (1:1)

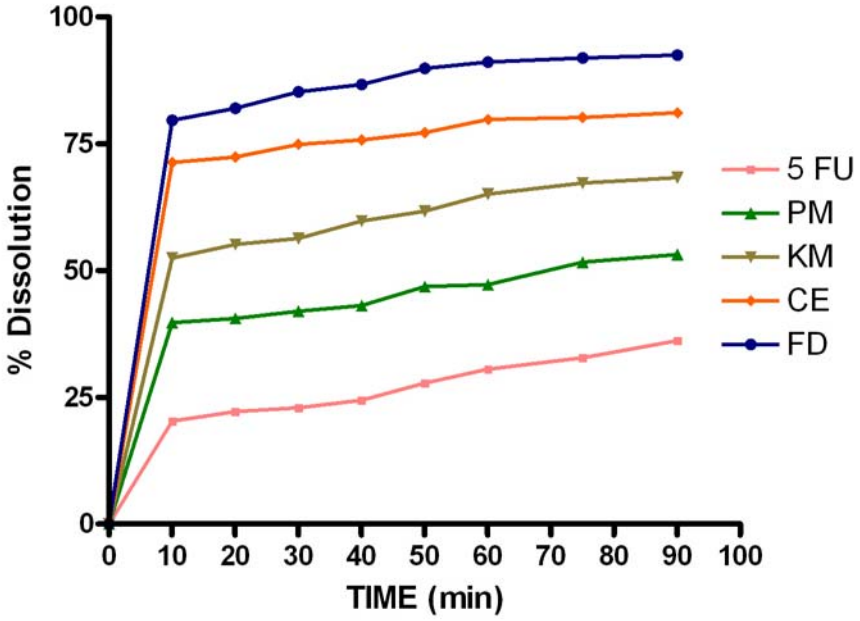
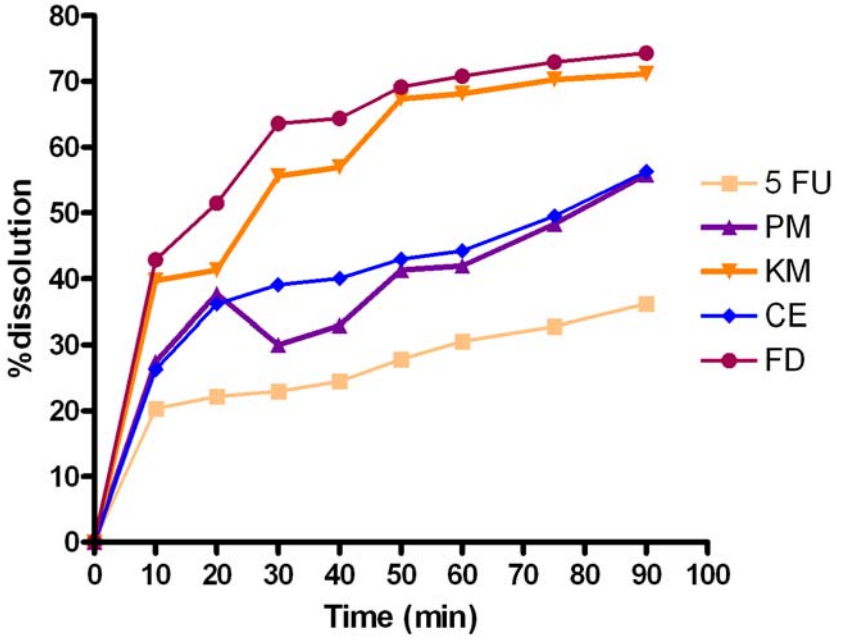


Fig 5. Dissolution profile of 5-fluorouracil and its complexes (1:2)



DIFFERENTIAL SCANNING CALORIMETRY GRAPHS OF DRUG AND COMPLEXES

Fig 3a: DSC of 5Fluorouracil

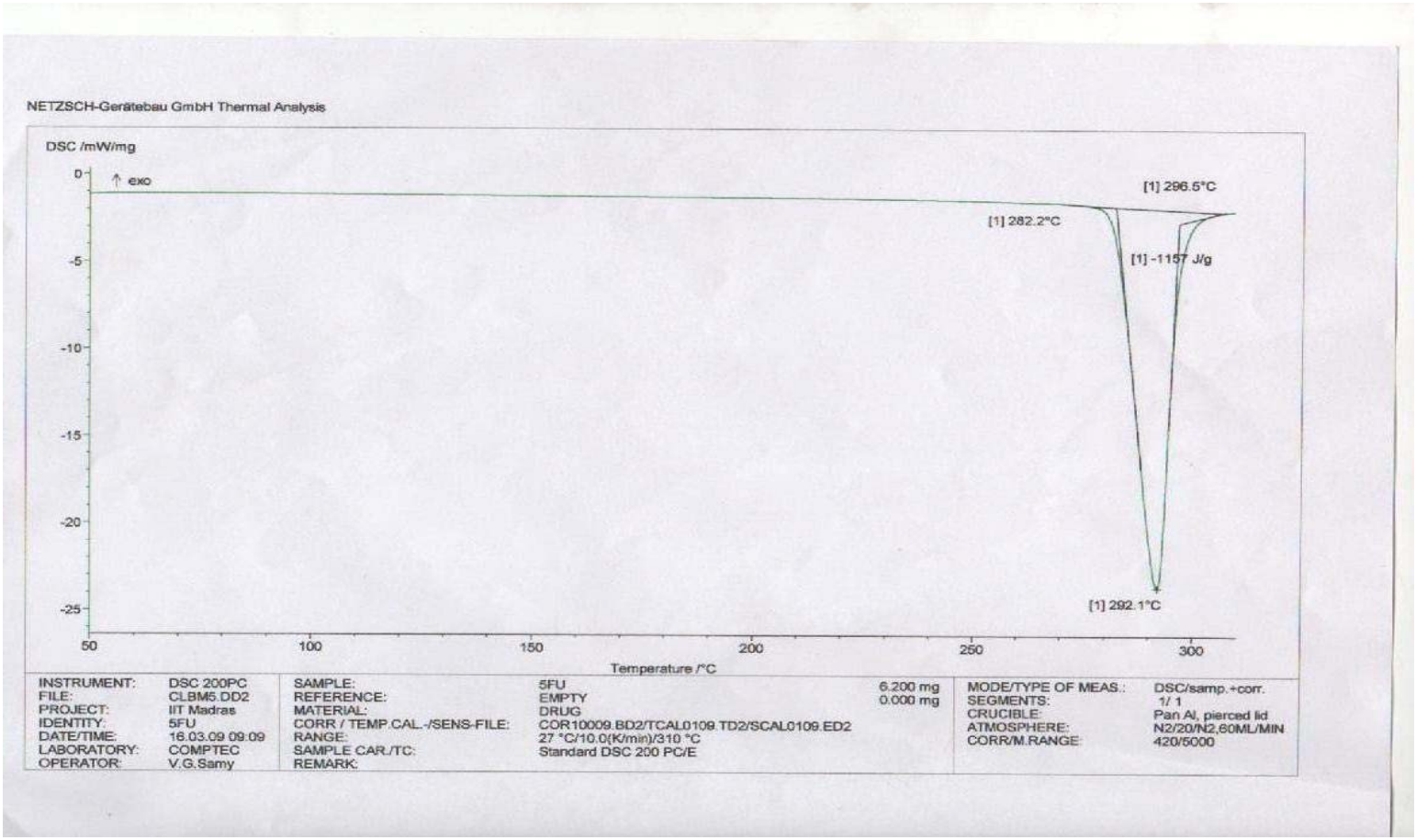


Fig 3b: DSC of β Cyclodextrin

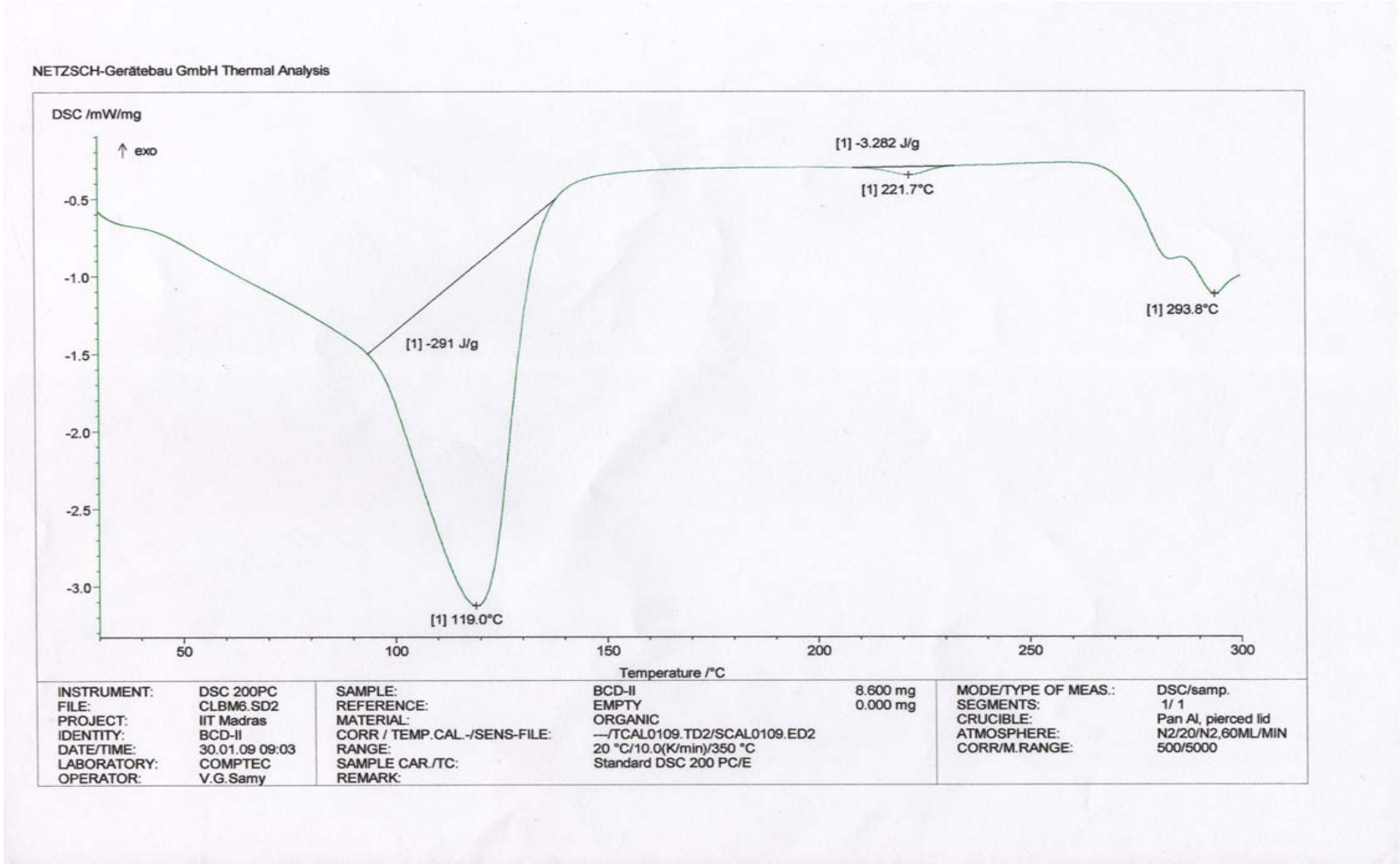


Fig 3f: DSC of Freeze Drying Method

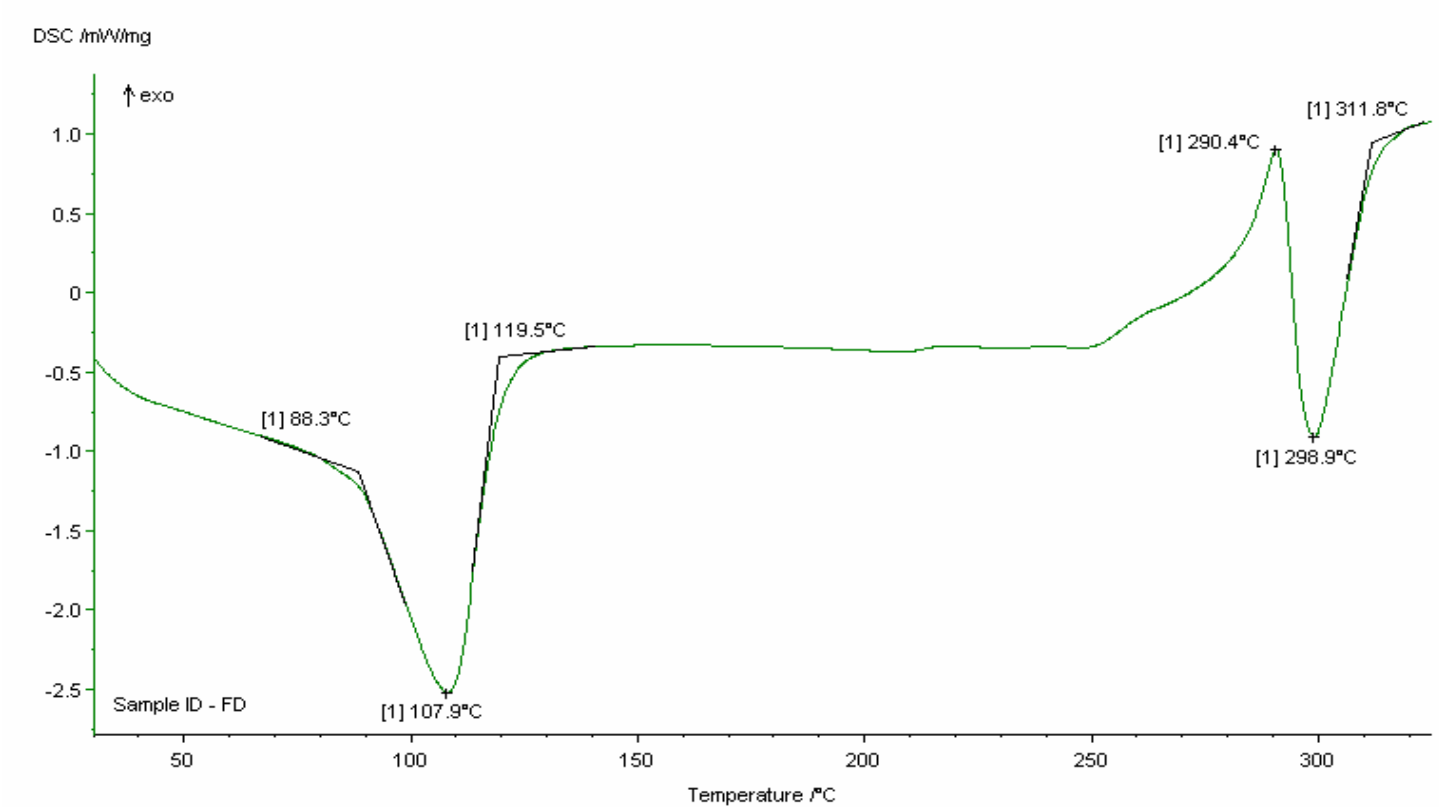


Fig 3e: DSC of Kneading Method

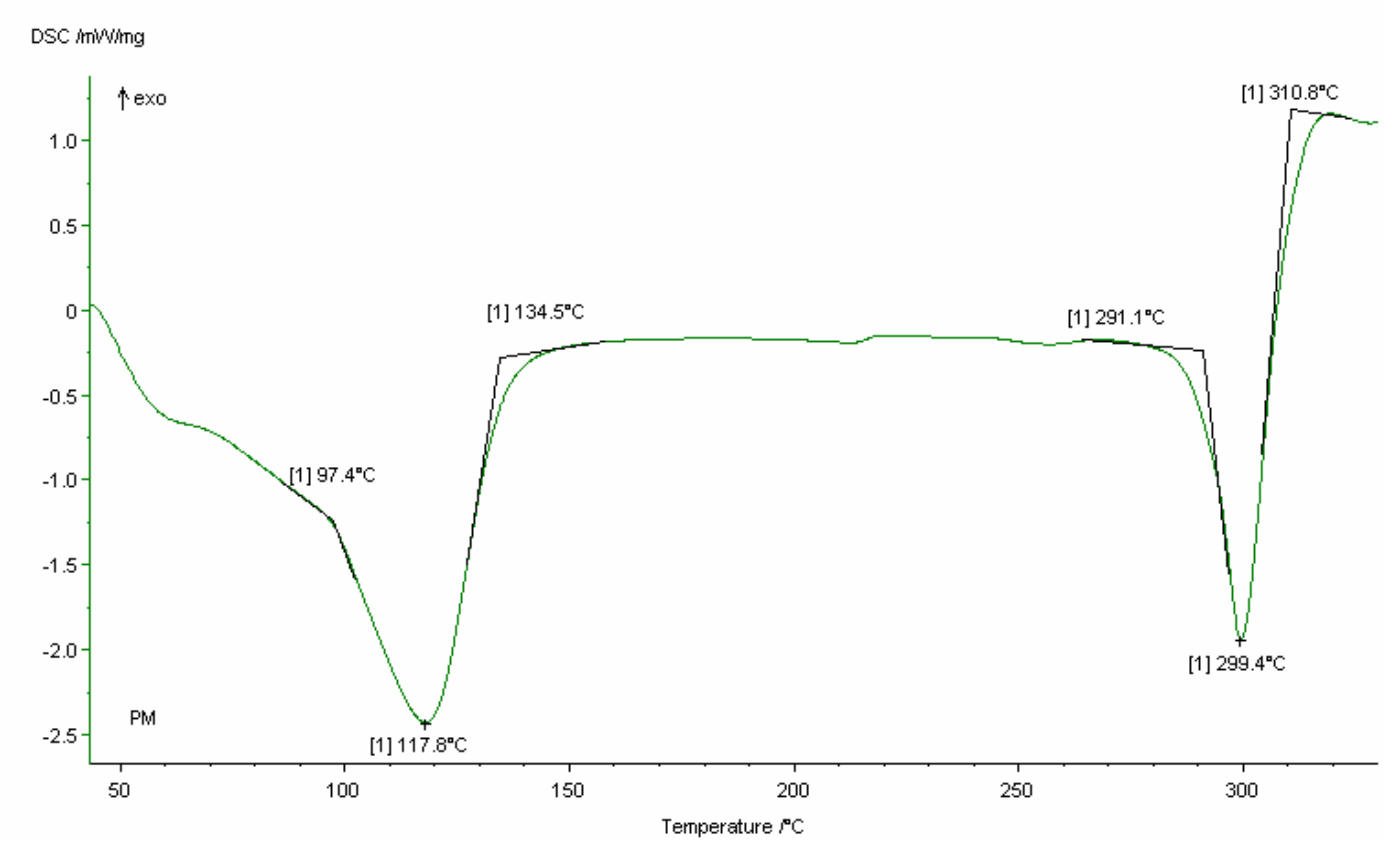


Fig 3c: DSC of Physical Mixture Method

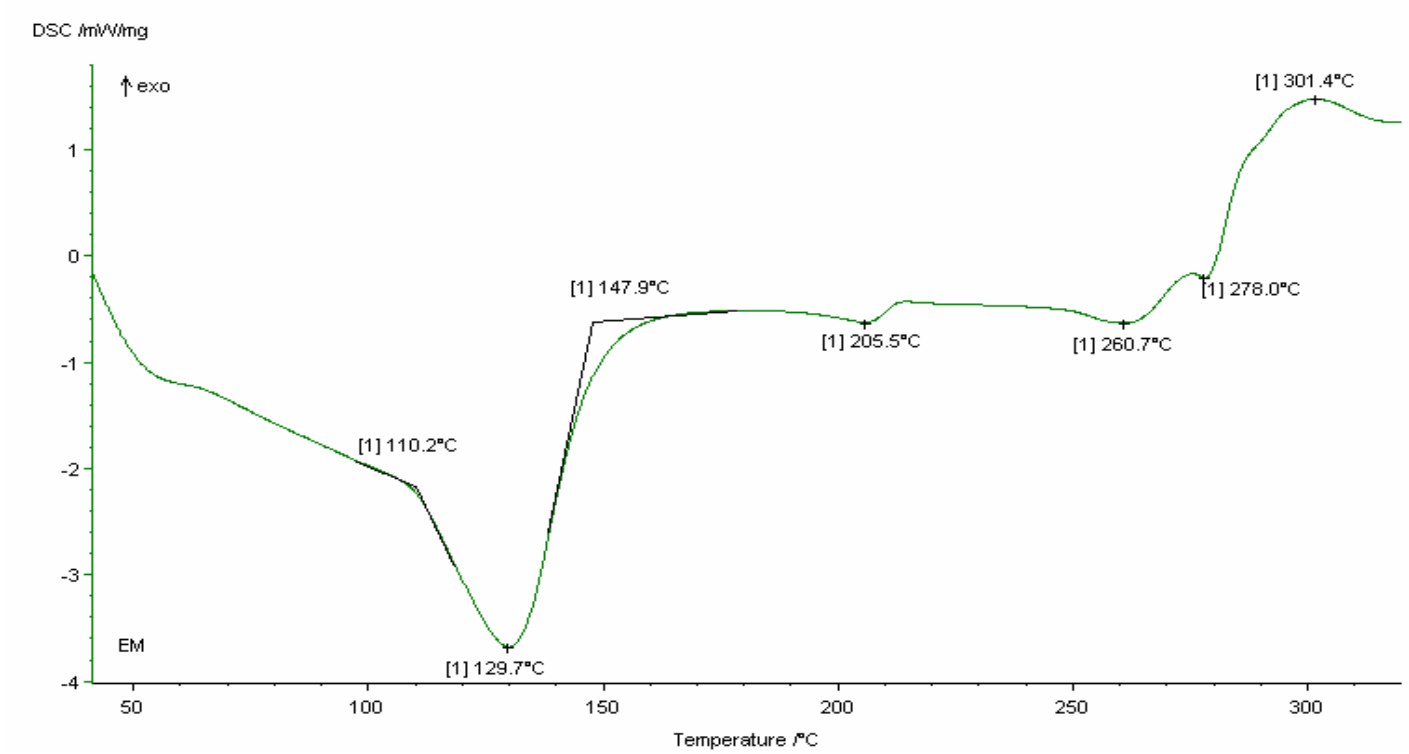


Fig 3d: DSC of Co Evaporation Method

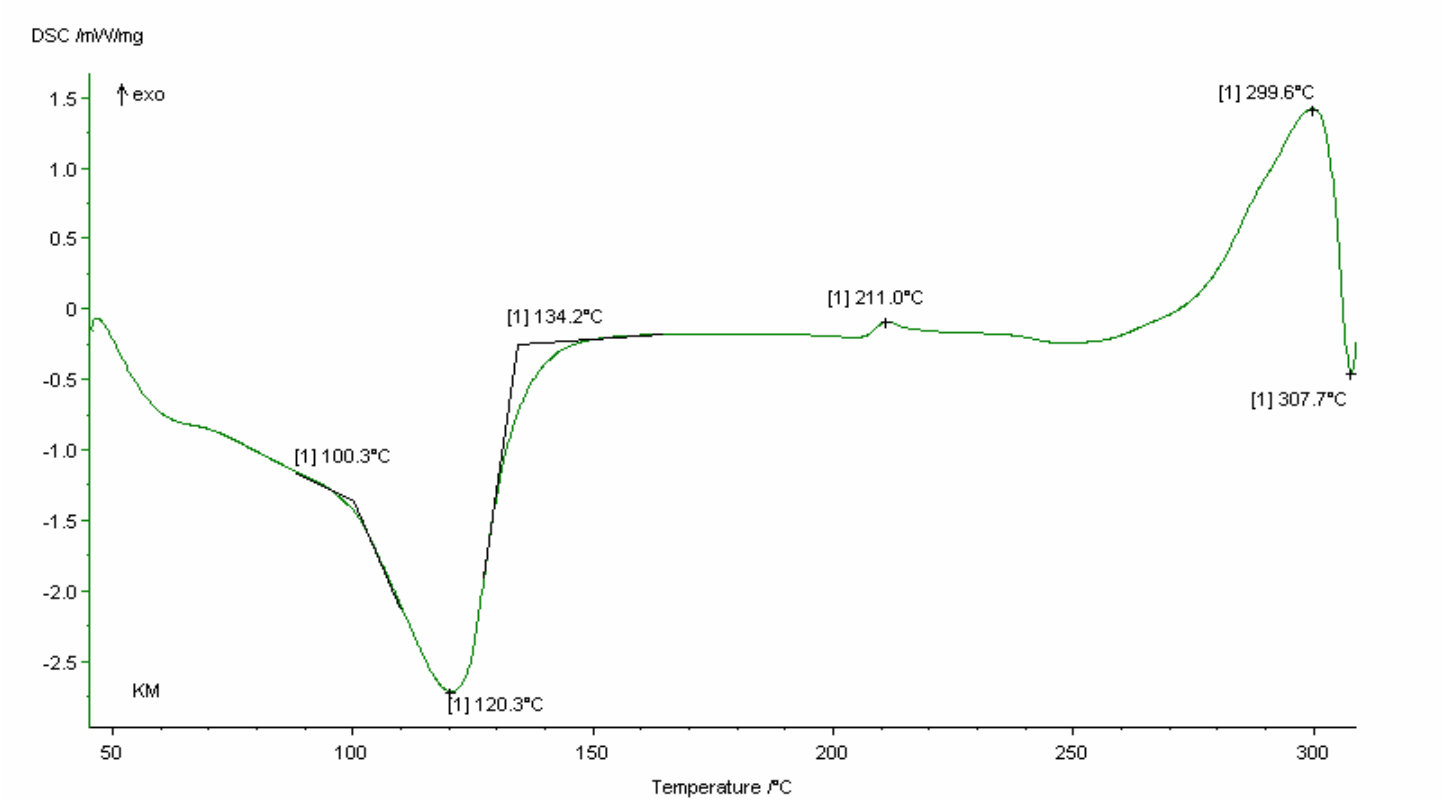
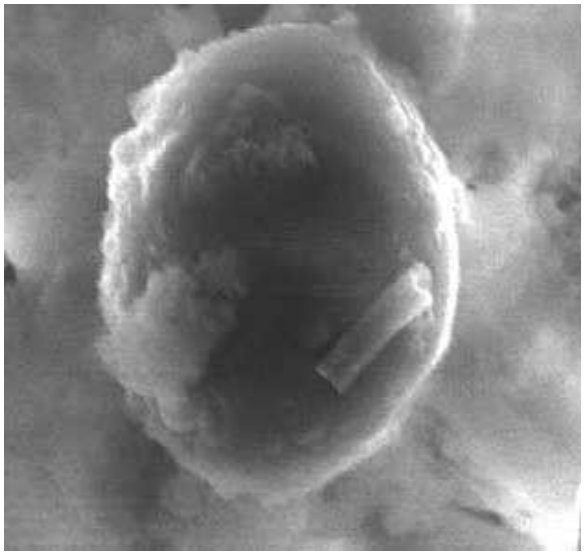


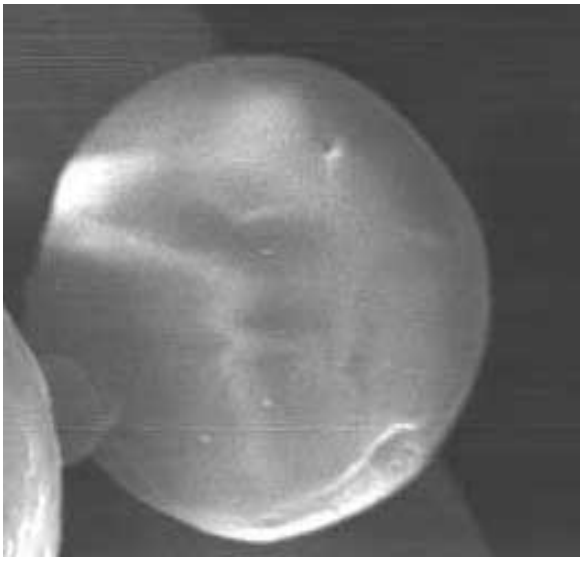
Fig: 7.5 SEM Photomicrographs of Different Formulations

FORMULATION M5

FORMULATION M6



FORMULATION M7



FORMULATION M8

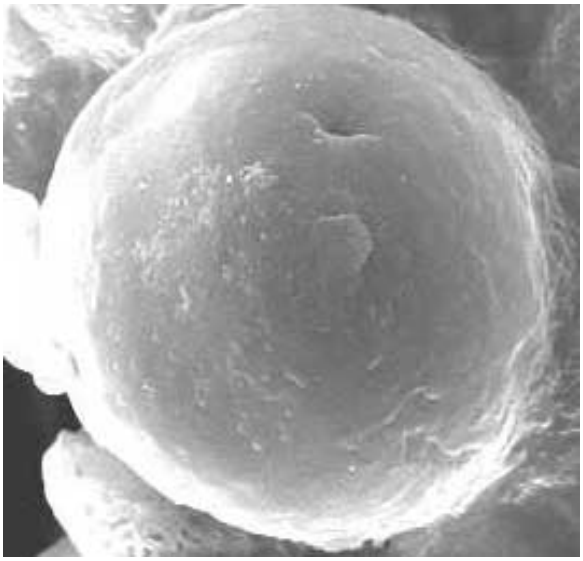
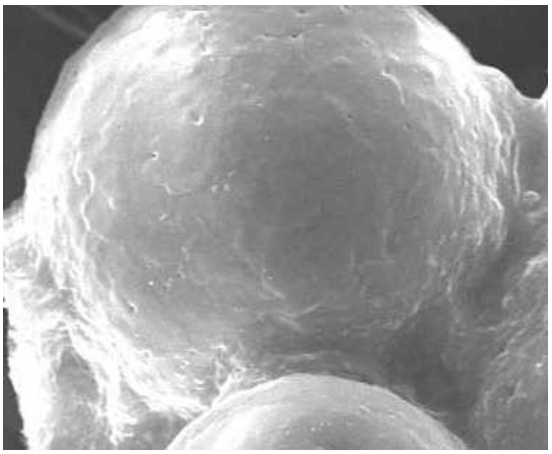


Fig: 7.5 SEM Photomicrographs of Different Formulations

FORMULATION M9



FORMULATION M11

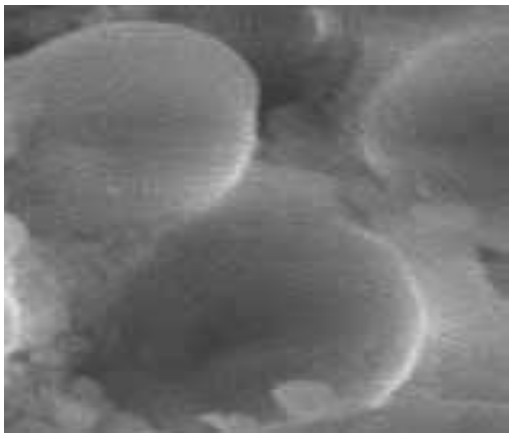
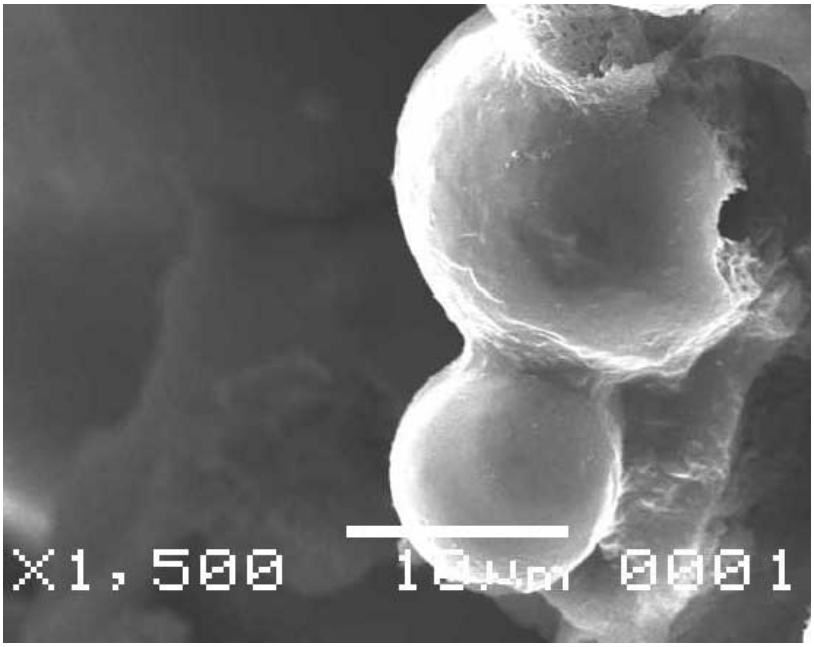
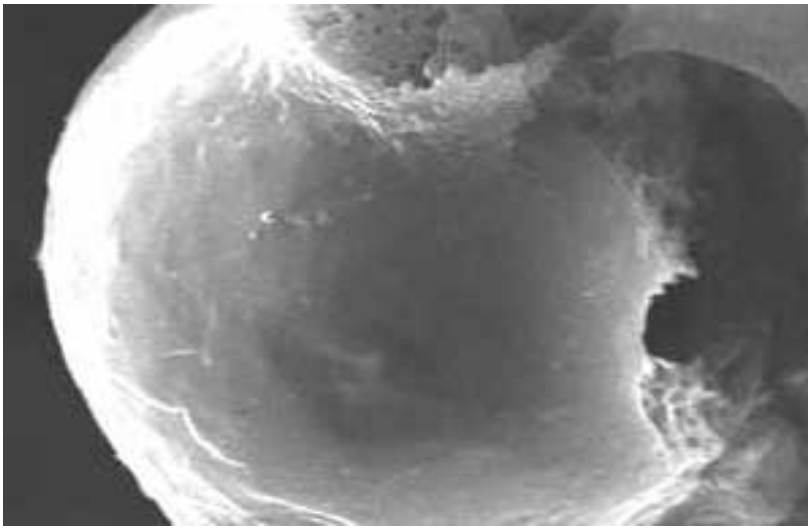


Fig: 7.6a SEM Photomicrographs of Formulation showing the hollow nature of the microsphere





FORMULATION AND EVALUATION OF FLOATING ORAL CONTROLLED RELEASE TABLETS OF 5 FLUOROURACIL

MATERIALS AND METHODS

MATERIALS USED

S.NO	MATERIALS	MANUFACTURER / SUPPLIERS
1	5 FLUORO URACIL	Biochem Pharmaceuticals Ltd.
2	HPMC K-100	Rolex Laboratories
3	HPMC K4M	Rolex Laboratories
4	HPMC K–50	Rolex Laboratories
5	HPMC KV-600	Rolex Laboratories
6	Sodium Bicarbonate	S.D Fine Chemicals Ltd
7	PEG – 6000	Merck India Ltd.
8	Hydrochloric Acid	S.D Fine Chemicals Ltd
9	Dichloro Methane	Merck India Ltd.
10	Acetonitrile	S.D Fine Chemicals Ltd
12	Barium sulphate	S.D Fine Chemicals Ltd
13	Magnesium Stearate	S.D Fine Chemicals Ltd
14	Talc	S.D Fine Chemicals Ltd

EQUIPMENTS USED

S.NO	NAME OF EQUIPMENTS	COMPANY
1.	Digital balance	Mettler, Japan.
2.	Mechanical stirrer	Remi Motors .Ltd Mumbai.
3.	Sonicator	Enertech Electronic Pvt.Ltd, Chennai.
4.	Hot air oven	Minicon, Mumbai.
5.	Dissolution apparatus	ElectrolabTDT-08L, Mumbai.
6.	pH Meter	E.I.Instruments, Chennai.
7.	UV Spectrophotometer	Shimadzu,UV-1601 Japan
8.	HPLC	Shimadzu V.P.series.
9.	FTIR	Boman, Model no MB 104,Canada.
10.	Humidity chamber	Sigma Instruments, Mumbai.
11.	Centrifuge	Remi Motors.Ltd Mumbai.
12.	Hardness tester	M.C. Dalal Agencies
13.	Vernier Calipers	Mitutoyo ,Japan
14.	Punch tablet machine	Cadmach ,Mumbai
15.	X-ray	Simco, Japan
16.	Friabilator	Electrolab, Mumbai.

LIST OF ABBREVIATIONS

5 FU		5 fluoro uracil
secs	-	Seconds
GRS	-	Gastro Retentive System
%	-	Percentage
µg	-	Microgram
IPA	-	Iso Propyl Alcohol
mg	-	Milligrams
nm	-	Nanometer
S.E	-	Standard Error
Fig.	-	Figure
PEG	-	Polyethylene glycol
e.g.		Example
FTIR	-	Fourier Transform Infra Red
UV	-	Ultraviolet Spectroscopy
ml	-	Milliliter
ºC	-	Degree centigrade
Rpm	-	Revolutions per minute
h	-	Hours
IP	-	Indian pharmacopoeia
HPMC	-	Hydroxy propyl methyl cellulose
USP	-	United states pharmacopoeia

OPTIMIZATION OF TABLET PROCESS PARAMETERS:

Optimization of tablet Formulae

Based on the results of the previous chapter, it was decided to formulate the gastro retentive systems with β cyclodextrin complexed drug prepared by freeze drying technique in the ratio of 1:1, which showed a higher dissolution profile when compared to other complexes.

Three different formulae were considered in order to optimize thickness, weight, and pressure so as to obtain floating property and required hardness.

Table: 6.1

S.No	INGREDIENTS	QUANTITY (mg)		
		Formula I	***Formula II***	***Formula III***
1	5 fluoro uracil	100	100	100
2	HPMC	100	150	200
3	Poly Ethylene Glycol 6000	25	50	75
4	Sodium Bicarbonate	50	75	100
5	PVP K-30	30	45	60
6	Lactose	50	75	100
7	Magnesium Stearate	2.5	5	10
8	Talc	2.5	5	10
9	Total weight of the tablet	360	505	655

The powder mixture was granulated and compressed by using Cadmach rotary tableting machine.

Optimization of tablet weight

The tablets were prepared with 5 fluoro uracil complex, HPMC (1:1) ratio and other excipients at different weights.

The weights of the tablets were optimized by varying the tablet weight with thickness and pressure constant.

The different weights were 200, 400 and 600 mg. Ten tablets were prepared at each weight. The floating property and hardness of each group of tablets were determined.

Table: 6.2

Pressure (Ton)	**Thickness (mm)**	**Weight (mg)**	**Floating property**	**Hardness ($Kg/cm^{2)}$**
5.5	2.9	200	Dispersed	3.1
		400	Floating	4.4
		600	Sink to the bottom	7.0

Optimization of tablet Thickness

The tablets were prepared with 5 fluoro uracil complex, HPMC polymer and other excipients at different thickness.

The thickness of the tablets was optimized by varying the tablet thickness, within a narrow range at constant pressure and weight.

The different thickness employed were 2, 2.9 and 3.3 mm .Ten tablets were prepared at each thickness. The floating property of each group of tablets was determined.

Table: 6.3.

Pressure (Ton)	Weight (mg)	Thickness (mm)	Floating property	Hardness ($Kg/cm^{2)}$
6.5	400	2.2	Dispersed	3.00
		2.9	Floating	4.40
		3.3	Floating	5.5

Optimization of tablet pressure

The tablets were prepared with 5 fluoro uracil complex, HPMC polymer and other excipients at different pressure.

The compression pressure was optimized by varying the pressure, keeping the thickness and weight constant.

The different compression pressures employed were 5.5, 6.5, 7.0 ton. Ten tablets were prepared at each compression. The floating property and hardness of each tablet were determined.

Table: 6.4

Thickness (mm)	Weight (mg)	Pressure (Ton)	Floating property	Hardness ($Kg/cm^{2)}$
2.9	400	4.5	Dispersed	3.2
		5.5	Floating	4.4
		7.0	Sink to the bottom	6.5

GENERAL PROCEDURE FOR PREPARATION OF 5 FLUORO URACIL GRS TABLETS (FI –FXII)

The granules were prepared by **wet granulation method**[157] as per formulae given in the Table 6.5, (Hundred tablets for each formulation)

- The drug 5 fluoro uracil, complexed with β cyclodextrin (1:1, Freeze drying technique), hydroxy propyl methyl cellulose (HPMC) of various grades, poly ethylene glycol 6000, Sodium bicarbonate, were passed through mesh 40# separately and blended thoroughly.

- The wet mass was passed through sieve 16# and dried at 65°C for one hour to get the moisture content less than one.

- The blend was granulated with PVP K-30 solution, which was prepared by dissolving PVP K-30 in IPA.

- Magnesium Stearate and talc were passed through sieve 40# and blended with dried granules.

- The lubricated granules were compressed on Cadmach eight punch tablet machine for all formulations.

Table6.6: **FORMULATION OF 5 FU GRS TABLET – F IX A**

Repetition of FIX

S.NO	INGREDIENTS	QTY (mgs) For one tablet
1	5 fluoro uracil (complexed with β cyclodextrin)	100
2	Hydroxy propyl methyl Cellulose K 100	50
3	Poly Ethylene Glycol 6000	25
4	Sodium Bicarbonate	50
5	PVP K-30	30
6	Lactose	50
7	Magnesium Stearate	3
8	Talc	3

The granules were prepared by **wet granulation method** as per formulae given in Table 6.6,

The complexed drug 5 fluoro uracil, hydroxy propyl methyl cellulose K 100, poly ethylene glycol 6000, Sodium bicarbonate, were passed through mesh 40# separately and blended thoroughly.

- The wet mass was passed through sieve 16# and dried at 65°C for one hour to get the moisture content less than one.
- The blend was granulated with PVP K-30 solution, which was prepared by dissolving PVP K-30 in IPA.
- Magnesium Stearate and talc were passed through sieve 40# and blended with dried granules.

CHARACTERIZATION OF 5 FU GRS GRANULES

Since tablets are made from granules hence it is necessary to evaluate the properties of granules, which may affect the final tablet characteristics.

Determination of Bulk and Tapped Density

An accurately weighed quantity of the granules (w) that was previously passed through # 40 was carefully poured into the graduated cylinder and the volume (v_o) was measured. The graduated measuring cylinder was tapped for 100 times and after that, the volume (v_f) was measured and continued the operation till the two consecutive readings were equal. Bulk density and tapped density determines the floating capacity of the formulation. The bulk density and tapped density were calculated using the formulas and the values are shown in Table 6.7 and in Fig.6.1 and 6.1a respectively.

Bulk density = w/v_o

Tapped density= w/v_f

Angle of Repose

The angle of repose represents flow property of granules. The angle of repose can be determined by fixed funnel method and the value is given in the Table 6.7. In this method, a funnel was clamped with its tip at a required height, above a graph paper, that was placed on a flat horizontal surface The Powder was allowed to flow carefully through the funnel until apex of the conical pile just touches the tip of the funnel. The radius of the pile was determined. The angle of repose[157] was calculated as

$$\theta = \tan^{-1} h/r$$

Where

θ - Angle of repose

h - height of the pile

r - radius of the pile

Compressibility Index

Compressibility is an important measure that can be obtained from the bulk and tapped densities. In theory, the less compressible a material, the more flowable it is. A material having values of 20 to 30% is defined as the free flowing material. The compressibility of the powder was calculated by determining the Carr's index and value are shown Table 6.8 and in Fig.6.2 respectively.

$$\text{Carr's index } \% = 100(v_o - v_f)/v_o$$

Loss on Drying

The granules were dried at a pressure below 5mm of Hg for 3 h and weight loss on drying was determined and the value is shown in the table 6.8 and fig 6.3 respectively.

CHARACTERIZATION OF 5 FU GRS TABLET (FI – FXII)

Tablet Size

Thickness of the tablet was measured by using Vernier caliper in mm. Thickness of fabricated tablets (F I - FXII) is presented in Table 6.9

Hardness test

Hardness test was carried out by using Monsanto hardness tester. Hardness of fabricated tablets (F I - FXII) is presented in Table 6.9 and in Fig.6.5 respectively.

Friability test

Friability of the tablets was tested using Roche friabilator. Loss of less than 1% in weight is considered to be acceptable. The weight of 10 tablets was noted initially (W1) and placed in the friabilator at 20 rpm. The tablets were reweighed and noted as (W2). The difference in the weight is noted and expressed as percentage. I.P official limit is not more than 1%. Friability[157] of fabricated tablets (F I - FXII) is shown in Table 6.9 and in Fig.6.4 respectively.

$$\text{Percentage Friability} = (W1 - W2)/W1 * 100$$

Weight variation test

Twenty tablets were selected at random and the average weight was determined. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in table and none deviates by more than twice the percentage.

IP official limit[155] of 5 FU GRS formulations (FI – F XII) percentage deviation is ± 5 %.

S.NO	Average weight of tablet	Percentage
1	80 mg or less	± 10 %
2	More than 80 mg and less than 250 gm	± 7.5 %
3	250 mg or more	± 5 %

The average weight and percentage deviation of (F I - FXII) are presented in Table 6.9.

Buoyancy determination

In practice floating time and buoyancy lag time was determined by using beaker[186,187] containing 100 ml of SGF, which was maintained at 37 ºC. The time required for the tablet to rise to the surface of the medium was determined as Buoyancy Lag time and the duration of which the tablet floats on the surface of the medium was noted as the floatation time. Results (FI – FXII) are presented graphically in Fig. 23, 24 and in Table 15, 16 respectively.

Drug content (assay)

Drug content[155] of the tablets were determined by using UV visible spectrophotometer and were presented in Table 6.12.

10 tablets were taken and powdered. The tablet powder equivalent to 100 mg of 5 fluoro uracil was accurately weighed and transferred to 100 ml volumetric flask and the volume was made up to 100 ml with SGF. 1ml of the aliquot was further diluted to 100 ml with SGF. The absorbance was measured at 266 nm.

***In vitro* Dissolution of Fabricated Tablets** (FI - FXII)

Dissolution of tablets was assessed using standard USP Dissolution apparatus (paddle) in 900 ml of SGF (pH 1.2). The stirring speed of 100 rpm was used. Three tablets were taken in each batch and a temperature of 37 ºC was maintained throughout the experiment.

Dissolution studies were carried out for 12 h. Samples of 10 ml were taken at intervals of 1h, 2h, 3h, 4h, 5h, 6h, 7h, 8h, 9h, 10h and 12h. After collecting the sample, the dissolution medium was replenished with the same volume of fresh medium. Samples were analyzed spectrophotometrically at 266 mm. The results were shown in table 6.13 – 6.24 and in Fig.6.8 – 6.11 respectively.

REFABRICATION AND EVALUATION OF F IX TABLETS

The formulation 5 FU GRS F-IX showed a good buoyancy lag time (25sec), duration of floating (24 h) with constant rate of release of drug, in controlled manner, similar to zero order kinetics. Hence 5 FU GRS F-IX was chosen as the best formulation. **Tablets of FIXA (batch 1) and FIX B (batch 2) were prepared again, based on the prototype formulation FIX, and evaluated to assess the reproducibility.**

Physical characteristics of the formulation 5 FU GRS F IXA & FIX B

Physical characteristics such as tablet size, hardness, friability, weight variation and drug content were evaluated and tabulated in Table 6.25.

Buoyancy determination:

The floating time and buoyancy lag time of 5 FU GRS F -IXA & F- IX B was determined by using beaker containing 100 ml of SGF, which was maintained at 37 ºC. The Time required to float was noted as buoyancy lag time and the duration for which the dosage form floated was noted as the floating time. Buoyancy lag time and duration of floating were compared at 25, 50, &100 rpm. The values were given in Table 32 and graphically represented in Figure 6.12 and 6.12a and fig 6.16a - 6.16d respectively.

***In vitro* dissolution profile of selected 5 FU GRS tablets (F-IX, F-IXA)**

Tablets dissolution was assessed using USP dissolution apparatus with 900ml of SGF. The stirring speeds of 25, 50& 100 rpm for the paddle apparatus were used. Three tablets were taken in each test and a temperature of 37± 1ºC was maintained throughout the experiment.

Dissolution studies were carried out for 12 h. Samples of 10 ml were taken at interval of 1h, 2h, 3h, 4h, 5h, 6h, 7h, 8h, 9h, 10h and 12h. After collecting the sample, the dissolution medium was replenished with the same volume of fresh medium. Samples were analyzed spectrophotometrically at 266 mm. The results were shown in table 6.27 and 6.27a and graphically in Fig 6.13 and 6.14 respectively.

The Scanning Electron Microscope (SEM) photomicrographs were taken for the formulation after one hour of dissolution and 12 hour of dissolution, to study the release pattern

Drug-excipients interaction study of selected formula by Infra red spectroscopy

Infra red spectra of formulation IX were recorded on FTIR spectrophotometer using KBr disc method. The absorption maxima in spectrum obtained with the sample being examined correspond to positive and relative intensity to those in the IR spectra of 5 fluoro uracil. The result is depicted in Fig 13 respectively.

Kinetic Studies (Mathematical model)

The drug release data of 5FU were fitted to models representing higuchi's (Cumulative percentage of drug released Vs square root of time), zero order (cumulative percentage of drug released Vs time), first order(log % unreleased Vs time and korsmeyer's equation (log cumulative percentage of drug released Vs time) kinetics to know the release mechanisms. The data were processed for regression analysis using MS Excel Statistical Function.

Equations[188,189] included are –

a. Zero order

$\%R = Kt$ $\quad t \rightarrow$ time; $\quad K \rightarrow$ Rate constant

b. First order

   Log % unreleased 0= Kt / 2.303

c. Matrix (Higuchi matrix)

   $\%R = Kt^{1/2}$ K = Higuchi dissolution constant

d. Peppas – Korsmeyer equation

   $\% R = Kt^{n}$

   Or Log %R = log K + n log t

   Where 'n' value is used to characterize different release mechanisms

After fitting into these models(Fig 6.15), the selection was based on the comparison of higher determination coefficient (r^2).

RESULTS AND DISCUSSION

The present study was undertaken to fabricate 5 fluoro uracil (5 FU) gastro retentive systems and to evaluate its characteristics. Evaluation of the product is a tool to ensure the performance characteristics required for the particular dosage form and to control batch to batch quality. Falling in line with this, the following characteristics were studied.

The granules were prepared using the ingredients in Table 6.5 and the physical characteristics of the granules of various formulations (F-I to F-XII) were investigated. Twelve formulations were developed. The physical characteristics of the granules (F-I to F-XII) such as bulk density, tapped density, angle of repose, loss on drying, compressibility index [190-192] were determined. The results are given in the table 6.7 and 6.8 and in Fig. 6.1, 6.1a, 6.2 and 6.3. The bulk density and tapped density ranged from 0.471 to 0.617 and 0.506 and 0.676 respectively. The percentage compressibility index was below 25, indicating good flow properties.

All the granules (F-I to F-XII) were found to be free flowing and their angles of repose were below 30.

Best Optimized formula from the three suggested ratios, for preparing the floating tablet by varying Weight, Thickness, Hardness, and Pressure was investigated. The best formula should posses the minimum buoyancy lag time (within few minutes) as well as maximum floatation time (more than 12h). The optimized values are presented in the Tables 6.1- 6.4 respectively. The selected formula was used for further tablet formulations (F-I to F-XII) using HPMC polymers of various grades like HPMC K100, HPMC K4M, HPMC KV600, and HPMC K50 in different ratios.

The physical properties of the tablets (F-I to F-XII) obtained by compressing the granules using Cadmach eight punches tablet machine were determined .The physical properties of 5 FU GRS tablets (F-I to F-XII) such as tablet size, hardness, friability and weight variation were determined and results are shown in the Table 6.9 and Fig 6.4 and 6.5 respectively. The hardness, friability, and weight variation of the formulations (F-I to

F-XII) was found to be within the limits specified in Pharmacopoeia. The hardness of the tablets ranged from 3.1 to 5.2 and percentage friability was in the range of 0.012 to 0.791. All the formulations showed reasonably good hardness value of approximately 4.1 kg/cm^2. Further, to strengthen these values, friability test values are also considered. The weight loss of less than 1% in friability test is considered as acceptable value for conventional tablet. This indicates that the tablets can withstand the mechanical shocks reasonably well during handling.

The drug content was estimated by UV spectroscopy for all formulations (F-I to F-XII). The values are shown in the Table.6.12. The drug content was found to be within a narrow range as specified in the Pharmacopoeia (95- 105 %) in all formulations. Drug content ranged from 97.69 -100.16 %

Buoyancy lag time and duration of floating was determined using USP dissolution test apparatus in SGF maintained at 37ºC and the results were represented in table6.10 and 6.11 & Fig 6.6 and 6.7 respectively. Buoyancy lag time of tablet F-IX was 24 seconds i.e. less than one minute which is the least value when compared to other tablets .The floatation time was found to be 24 hours except for tablet F-V.

Based upon the buoyancy lag time and floatation time, the formulation F-IX was selected as the best formulation. The dissolution studies of the formulation (F-I-to F-XII) were carried out in USP dissolution apparatus (paddle) in 900 ml SGF as dissolution medium. The reports are represented in the tables 6.13 – 6.24 and Fig 6.8 – 6.11 respectively. The dissolution studies of the formulation F-I,F-IV, F-VII, F-XI was below 55%. Whereas for F-II, F-V, F-VIII was below 70% respectively. The better release is due to viscosity difference between the grades of polymer. An optimum viscosity grade is suitable for floating dosage form.

From the results of *invitro* release study of formulations F I, FII and F III, it is evident that as the ratio of HPMC increases the release decreased. This behavior may be due to the fact that more amount of polymer always delays the release.

According to ford and co workers [193-195] and Xu and Sunada [196], the most important factor affecting the rate of release from HPMC matrices is the drug: HPMC ratio. An increase in

polymer concentration causes an increase in viscosity of the gel as well as the formation of a gel layer with a longer diffusional path. This could cause a decrease in the effective diffusion coefficient of the drug and therefore a reduction in the drug release rate[197].

From the *invitro* release studies, the formulation F IX was found to have maximum release.

Curve fitting analysis

According to the data the release of drug followed diffusion controlled mechanism from the formulation. Release of drug from matrix tablet containing hydrophilic polymers generally involves factors of diffusion. Diffusion is related to transport of drug from the dosage form in to the invitro fluid depending on the concentration. As the gradient varies the drug is released and the distance for diffusion increases. This could explain why the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred as square root kinetics or higuchi's kinetics. In the present study the invitro profiles of drug from all the formulations could be best expressed by Higuchi's equation as all the formulations showed linearity (r^2 : 0.9586- 0.9937 indicating that diffusion is the dominant mechanism of drug release with these formulations.

The tablets were found to follow zero order kinetics. The r^2 values of zero order are high when compared to r^2 values of first order. This states that the release of drug is concentration independent.

The mode of drug release from floating tablets was evaluated using the korsmeyer peppas method. The value of n fell within the range of 0.6838 to1.0546 for formulations F I to F VI. This denotes that the release is non fickian. But for other formulations (F VII to F XII) it was found to be around 0.50(0.5377 to 0.6150), showing that the release behavior follows fickian diffusion, in which the rate of dissolution medium uptake in to the polymer is largely determined by the relaxation of the polymer chains.

F IX was selected as the best formulation with zero order release with Higuchi type of diffusion showing fickian pattern. Formulations F IXA and F IXB were prepared

with the same constituents as that of F IX, to check the reproducibility. The tablets of F IXA and F IXB were again studied for the properties and performance. Immediately after manufacture F IXB was subjected to stability studies. The characters such as tablet size, hardness, friability, drug content and weight variation were determined and the results are given in Table 6.25. Buoyancy and floatation time for F IXA and F IXB were determined using dissolution apparatus in SGF (pH1.2) as medium at 25, 50 and 100 rpm. The results are compared in Table 6.26 and 6.26a and in the Fig 6.12 and 6.12a respectively.

Floating property was determined by Beaker method (invitro). The photographs of the floating tablets are presented in Fig 6.16a – 6.16 d respectively .The results showed that the tablet was floating up to 12 hours.

The dissolution studies were carried out for F IXB formulation in SGF at 25 (fed state), 50(fasted state) and 100 rpm. The results are shown in the table 6.27 – 6.29 and in Fig 6.13 and 6.14 respectively. The results showed more or less same release characteristics. From this it is confirmed that the results are reproducible. Comparison of the release profiles at different rpm denoting the fast and fed state condition states that the dissolution profile is less affected by the difference in rpm.

The photomicrographs of the formulation (fig 6.17 a and 6.17 b) after first and twelfth hour showed that the drug diffused out of the formulation by erosion and diffusion mechanism

The drug interaction between polymer and other excipients were determined by using FTIR spectrophotometer and by DSC studies. The results are shown in Fig. 2 - 16 respectively. No interaction was observed.

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Table 6.5: General Formula and Composition of Fluorouracil GRS Tablets

S.No	Active Ingredients	Formulation (Quantity in mg)											
		F I	**F II**	**F III**	**F IV**	**F V**	**F VI**	**F VII**	**F VIII**	**F IX**	**F X**	**F XI**	**F XII**
1	**5 FU (Complexed with β cyclodextrin)**	100	100	100	100	100	100	100	100	**100**	100	100	100
2	**HPMC KV600**	150	100	50	-	-	-	-	-	**-**	-	-	-
3	**HPMC K4M**	-	-	-	150	100	50	-	-	**-**	-	-	-
4	**HPMC K100**	-	-	-	-	-	-	150	100	**50**	-	-	-
5	**HPMC K 50**	-	-	-	-	-	-	-	-	**-**	150	100	50
6	**PEG 6000**	25	25	25	25	25	25	25	25	**25**	25	25	25
7	**Sodium bicarbonate**	50	50	50	50	50	50	50	50	**50**	50	50	50
8	**PVP K-30**	50	50	50	50	50	50	50	50	**50**	50	50	50
9	**Lactose**	50	50	50	50	50	50	50	50	**50**	50	50	50
10	**Magnesium stearate**	3	3	3	3	3	3	3	3	**3**	3	3	3
11	**Talc**	3	3	3	3	3	3	3	3	**3**	3	3	3

Physical characteristics of granules (F I – F XII)

Table: 6.7 **Determination of Bulk and Tapped Density**

Batch No	Bulk Density	Tapped Density	Angle of repose $\theta = \tan^{-} h/r$
FI	0.494	0.539	24°66'
FII	0.519	0.576	27°79'
FIII	0.474	0.506	23°78'
FIV	0.617	0.676	29°40'
FV	0.535	0.590	26°26'
FVI	0.494	0.529	24°19'
FVII	0.471	0.521	21°94'
FXIII	0.519	0.554	27°30'
FIX	0.495	0.543	29°48'
FX	0.508	0.575	26°57'
FXI	0.541	0.580	29°57'
FXII	0.506	0.551	28°84'

Fig : 6.1 Comparison of Bulk density of 5 FU Granules

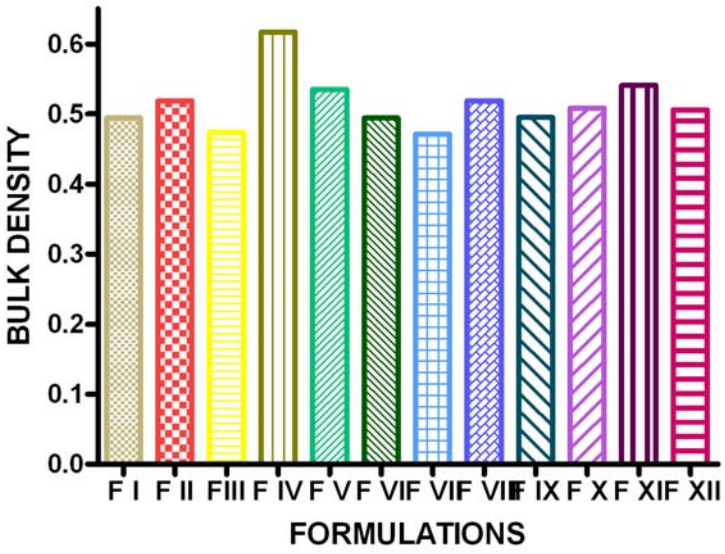


Fig : 6.1a Comparison of tapped density of 5 FU Granules

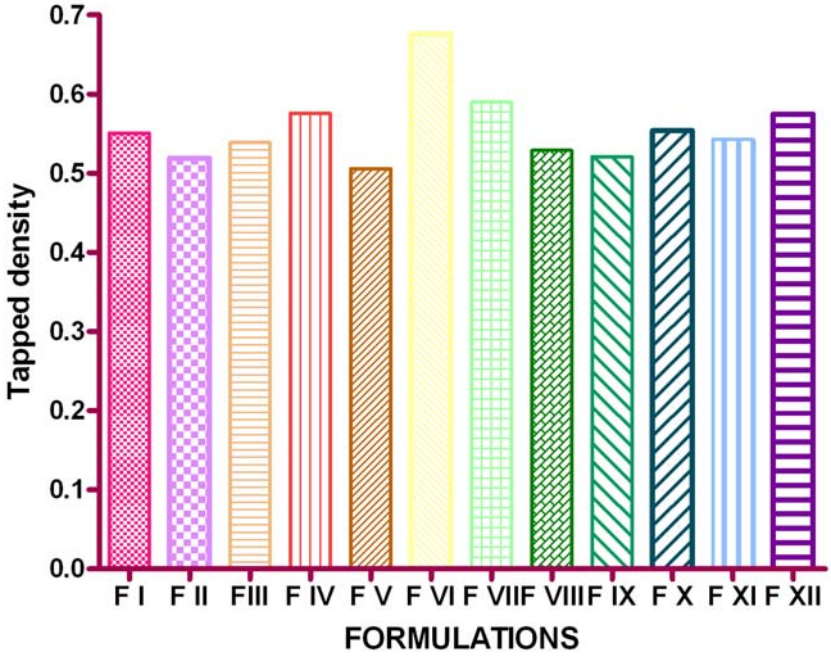


Table: 6.8 **Percentage Compressibility index of 5 FU GRS Granules (FI – FXII)**

S.No	Batch No	% Compressibility index	% Loss on drying
1	F I	8.40	0.07
2	F II	9.87	0.04
3	F III	6.40	0.05
4	F IV	8.64	0.03
5	F V	9.36	0.08
6	F VI	6.67	0.06
7	F VII	9.65	0.04
8	F VIII	6.23	0.09
9	F IX	8.91	0.02
10	F X	11.68	0.05
11	F XI	6.76	0.07
12	FXII	8.10	0.05

Fig: 6.2 Comparison of % compressibility index of 5 FU granules

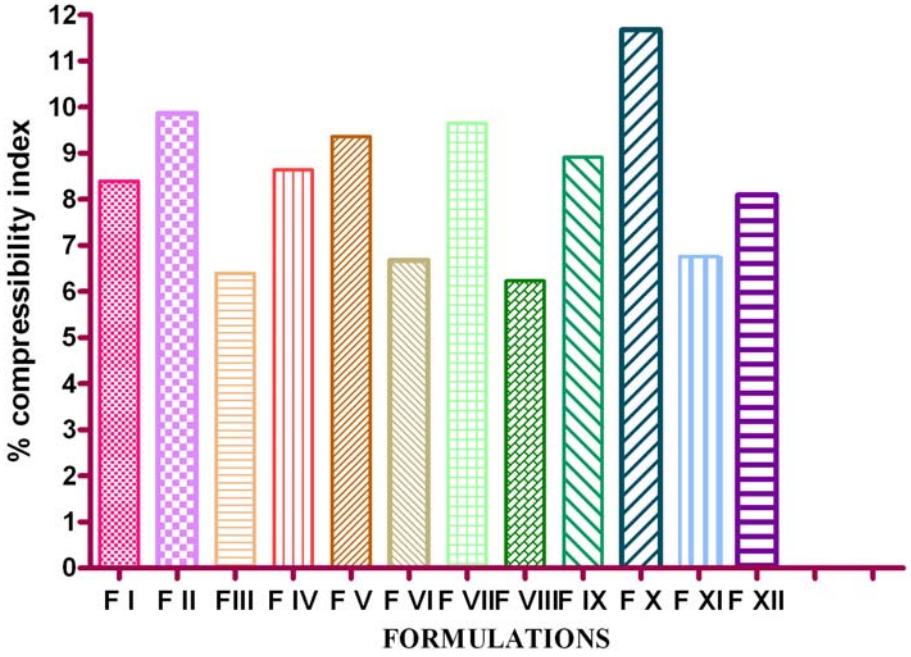


Fig: 6.3 Comparison of % loss on drying of 5 FU granules

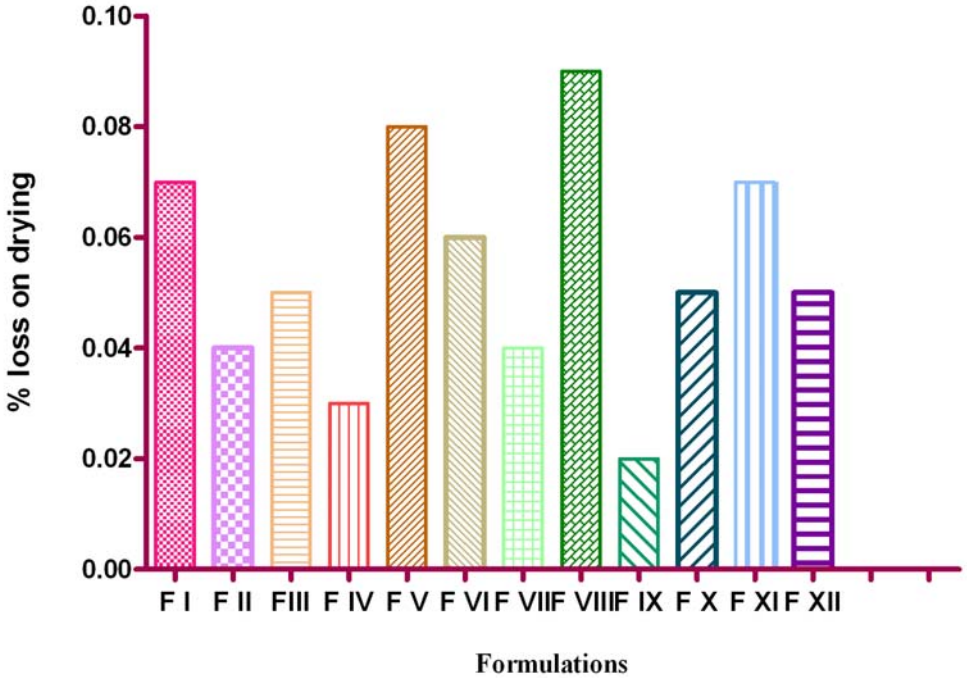


Table: 6.9. **Physical Characteristics of 5 Fluoro Uracil GRS Tablets (FI-FXII)**

Batch No	Weight Variation (mg±SD)	Friability (%)	Hardness (kp)	Thickness (mm)
F I	436±0.6	0.037	3.8	2.94
F II	385±1.2	0.043	4.1	3.40
FIII	340±1.2	0.081	3.1	2.84
F IV	441±1.8	0.088	3.4	2.34
F V	391± 1.9	0.049	4.2	3.63
F VI	342±1.6	0.550	3.2	2.98
F VII	437±1.5	0.063	3.9	3.69
F VIII	398±0.7	0.030	4.1	3.67
F IX	352±0.4	0.029	4.4	2.99
F X	452±0.8	0.012	4.1	3.14
F XI	382±1.1	0.791	4.2	2.93
F XII	345±2.1	0.016	3.8	2.94

Fig : 6.4 Comparison of physical characteristics of 5 FU tablets % Friability

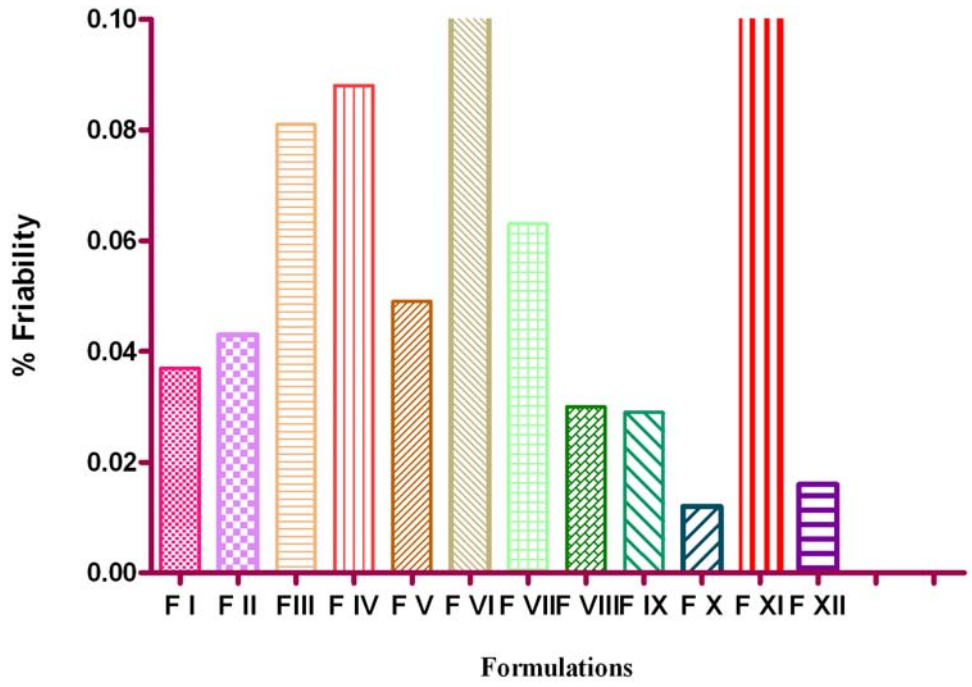


Fig : 6.5 Comparison of physical characteristics of 5 FU tablets - Hardness

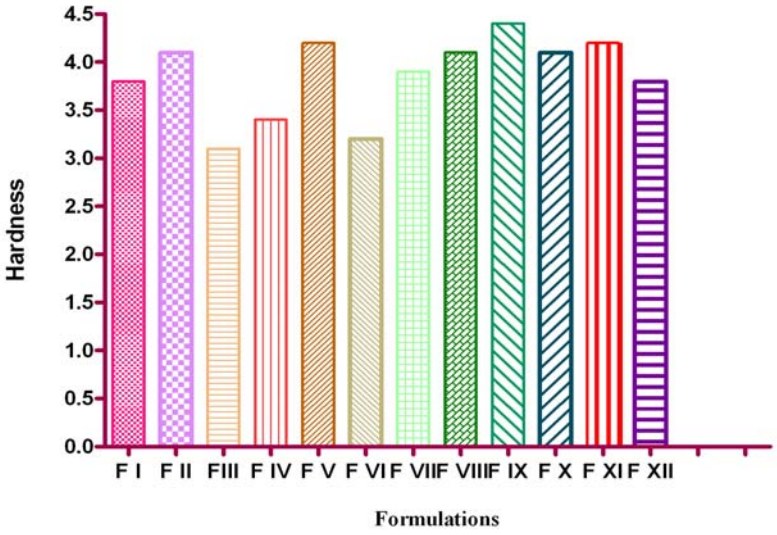


Table: 6.10 **Buoyancy lag time of 5 FU GRS tablets (FI – FXII)**

S.No	Batch No	Buoyancy lag time (sec)
1	F I	75
2	F II	41
3	F III	31
4	F IV	25
5	F V	39
6	F VI	61
7	F VII	44
8	F VIII	58
9	F IX	24
10	F X	123
11	F XI	175
12	FXII	95

Table: 6.11 **Floating duration of 5 FU GRS tablets (FI – FXII)**

S.No	Batch No	Floating duration (hrs)
1	F I	24
2	F II	24
3	F III	24
4	F IV	24
5	F V	20
6	F VI	24
7	F VII	22
8	F VIII	24
9	F IX	24
10	F X	24
11	F XI	24
12	FXII	24

Fig 6.6: Comparison of buoyancy lag time of 5 FU tablets

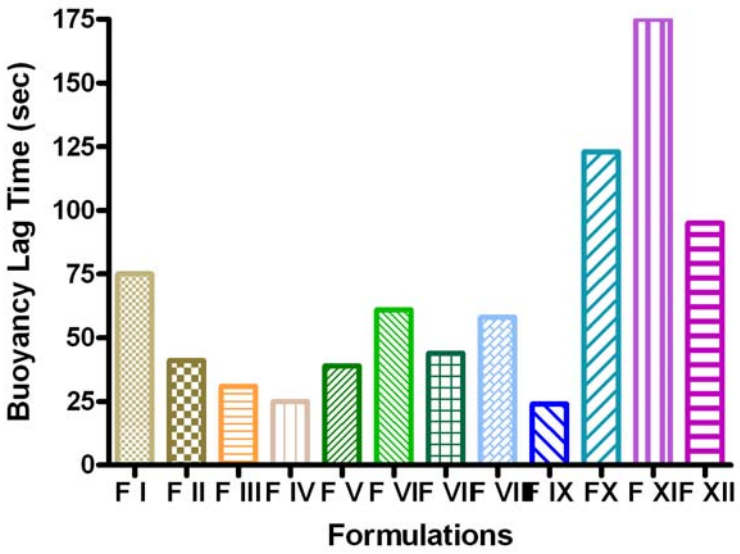


Fig 6.7: Comparison of floatation time of 5 FU tablets

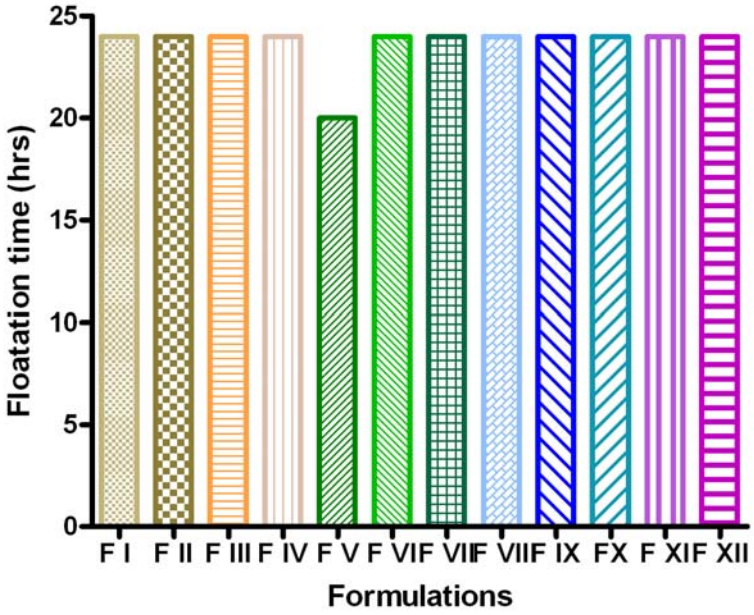


Table: 6.12. **Drug content of fabricated 5 Fluoro Uracil Floating Tablets (FI-FXII)**

S.No	Batch No	Amount of drug in the tablet (mg)			Percentage Drug Content (Mean ±SE)
		Tablet 1	Tablet 2	Tablet 3	
1	FI	100.2	100.4	100.6	100.4 ± 2.71
2	FII	100.6	101.0	101.1	100.9 ± 3.11
3	FIII	101.3	100.4	98.6	100.1 ± 1.69
4	FIV	103.8	100.0	100.2	101.3± 3.19
5	FV	100.2	101.2	100.9	100.76± 2.26
6	FVI	99.4	101.4	100.8	100.53± 4.05
7	FVII	99.9	101.5	99.6	100.33± 2.38
8	FVIII	101.2	99.4	98.6	99.73± 1.22
9	FIX	100.1	99.2	99.8	99.7 ± 1.04
10	FX	98.6	99.7	100.2	99.5± 3.06
11	FXI	101.2	100.2	100.1	100.5 ± 4.23
12	FXII	101.9	100.2	99.4	100.5 ± 2.79

***Table: 6.13. In Vitro* Dissolution Study of 5 FU Floating tablets (F-I)**

Medium : Simulated Gastric Fluid pH 2, 900 ml
Apparatus : USP basket
Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	4.40	3.96	3.94 ± 0.31
2	2	7.18	6.47	6.44 ± 1.26
3	3	10.31	9.28	9.24 ± 1.29
4	4	13.33	12.0	11.95 ± 2.36
5	5	18.59	16.73	16.66 ± 0.44
6	6	23.20	20.88	20.80 ± 0.55
7	7	34.16	30.75	30.63 ± 4.77
8	8	38.32	34.49	34.35 ±3.65
9	9	43.57	39.22	39.06 ± 2.52
10	10	52.38	47.14	46.95 ± 1.54
11	12	61.33	55.20	54.98 ± 0.25

Cumulative % Drug Release readings are mean ±SE of triplicate values.

Table: 6.14 *In Vitro* Dissolution Study of 5 FU Floating tablets (F II)

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket , Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	7.86	7.07	7.00 ± 0.17
2	2	10.49	9.44	9.36 ± 2.29
3	3	16.3	14.67	14.54 ± 2.60
4	4	21.58	19.42	19.25 ± 0.40
5	5	29.18	26.26	26.03 ± 1.38
6	6	35.52	31.97	31.68 ± 2.60
7	7	40.53	36.48	36.15 ± 0.59
8	8	48.14	43.33	42.94 ± 0.26
9	9	51.76	46.58	46.16 ± 1.34
10	10	57.22	51.50	51.04 ± 1.56
11	12	63.88	57.49	56.98 ± 0.42

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.15. In Vitro* Dissolution Study of 5 FU Floating tablets (F-III)**

Medium : Simulated Gastric Fluid pH 2, 900 ml
Apparatus: USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	9.0	8.10	8.09 ± 1.42
2	2	17.26	15.53	15.51 ± 2.21
3	3	23.33	21.00	20.98 ± 0.41
4	4	36.71	33.04	33.0± 1.48
5	5	45.11	40.60	40.56 ± 0.20
6	6	49.11	44.20	44.16 ± 2.11
7	7	55.9	50.31	50.26 ± 2.39
8	8	59.46	53.51	53.46 ± 0.44
9	9	65.85	59.27	59.21 ± 1.40
10	10	73.08	65.77	65.70 ± 1.21
11	12	81.79	73.61	73.54 ± 0.40

Cumulative % Drug Release readings are mean ±SE of triplicate values.

Release kinetics of F1

	First order Release	Higuchi release	Korsmeyer peppas Release	Zero order release
Slope	4.442976	16.71454549	1.054672573	-0.00543
Correlation	0.976955	0.942892981	0.98176302	-0.99873
r^2	0.95444	0.889047173	0.963858627	0.997469

Release kinetics of F II

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	5.2825	20.17566729	0.916538059	-0.00583
Correlation	0.995911	0.97583504	0.985431347	-0.99769
r^2	0.991839	0.952254026	0.971074939	0.995393

Release kinetics of FIII

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	6.767143	26.47879424	0.942127136	-0.00648
Correlation	0.988443	0.992228885	0.995027066	-0.9961
r^2	0.977019	0.984518161	0.990078862	0.992206

Fig 6.8: *Invitro* dissolution profile of formulations F I - FIII

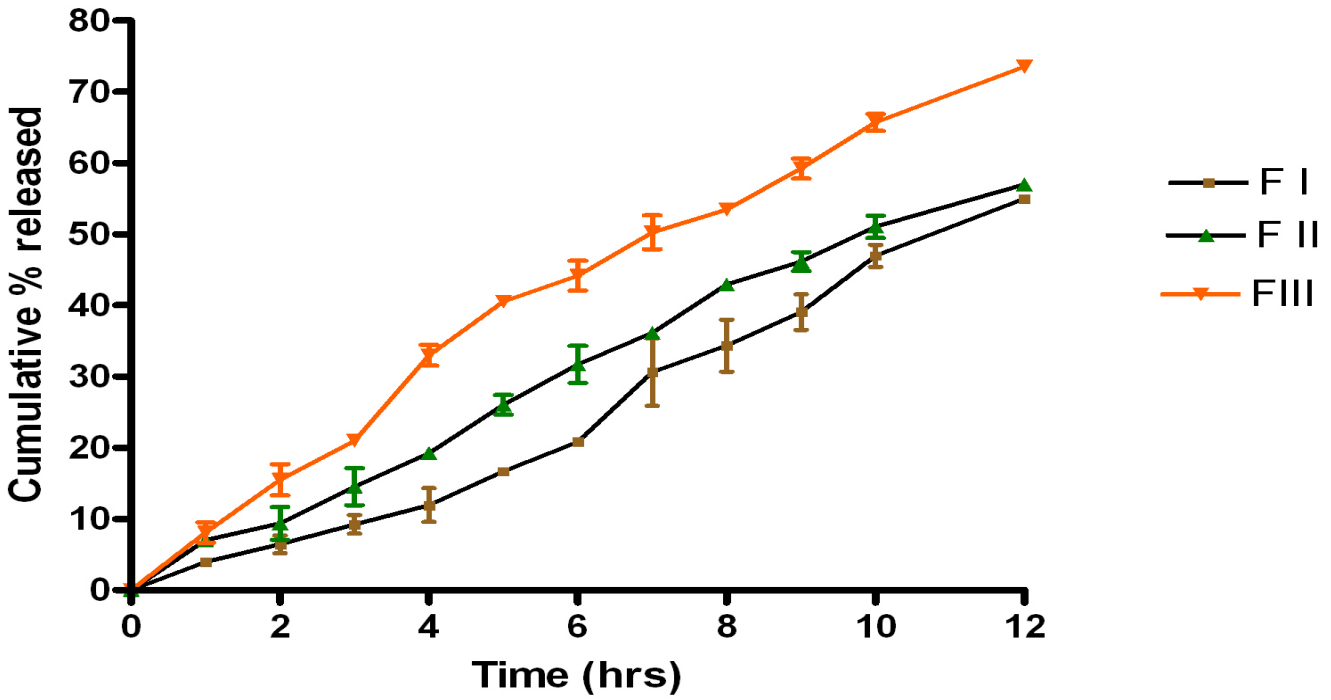


***Table: 6.16 In Vitro* Dissolution Study of 5 FU Floating tablets (F-IV)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus: USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	4.32	3.89	3.86 ± 1.11
2	2	6.09	5.48	5 ± 1.31
3	3	7.49	6.74	6.90 ± 0.12
4	4	10.23	9.21	9.39 ± 1.49
5	5	12.68	11.41	11.46 ± 2.50
6	6	15.37	13.83	13.88 ±0.36
7	7	16.53	14.88	14.79 ± 0.53
8	8	18.98	17.08	17.31 ± 1.47
9	9	21.69	19.53	19.77 ± 0.35
10	10	28.36	25.12	25.65 ± 2.43
11	12	35.87	32.38	32.58 ± 1.32

Cumulative % Drug Release readings are mean ±SE of triplicate values

***Table: 6.17 In Vitro* Dissolution Study of 5 FU Floating tablets (F-V)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus: USP basket, Rpm: 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	11.24	10.12	10.0 ± 1.29
2	2	15.93	14.53	14.34 ± 2.61
3	3	22.06	19.85	19.59 ± 0.26
4	4	24.57	22.11	21.83 ± 2.52
5	5	31.8	28.62	28.25 ± 1.35
6	6	36.19	32.57	32.15 ± 1.52
7	7	42.08	37.87	37.38 ± 2.41
8	8	44.98	40.48	39.96 ± 1.56
9	9	49.5	44.55	43.98 ± 0.50
10	10	53.08	47.77	47.16 ± 1.45
11	12	54.7	49.23	48.60 ± 0.29

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.18 In Vitro* Dissolution Study of 5 FU Floating tablets (F-VI)**

Medium : Simulated Gastric Fluid pH 1. 2, 900 ml
Apparatus : USP basket , Rpm100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	7.04	6.34	6.31 ± 2.23
2	2	12.0	10.8	10.76 ± 1.51
3	3	17.78	16.0	15.95 ± 0.31
4	4	23.92	21.53	21.46 ± 5.45
5	5	28.80	25.92	25.83 ± 0.53
6	6	36.42	32.78	32.67 ± 0.17
7	7	43.18	38.86	38.73 ± 1.53
8	8	47.92	43.33	43.19 ±1.41
9	9	54.13	48.72	48.56 ± 0.55
10	10	64.44	59.5	59.32 ± 2.41
11	12	89.86	80.17	79.97 ± 1.61

Cumulative % Drug Release readings are mean ±SE of triplicate values.

Release kinetics of FIV

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	1.9775	7.610674707	0.758962894	-0.00485
Correlation	0.996463	0.983864177	0.986097099	-0.99979
r^2	0.992938	0.967988719	0.972387489	0.999578

Release kinetics of FV

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	4.393095	16.97683672	0.683818046	-0.00588
Correlation	0.997173	0.98860799	0.99361221	-0.99843
r^2	0.994354	0.977345759	0.987265224	0.996859

Release kinetics of FVI

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	5.387381	20.70344377	0.943764684	-0.00587
Correlation	0.998698	0.984615061	0.997661265	-0.99758
r^2	0.997398	0.969466818	0.995328001	0.995164

Fig: 6.9 *Invitro* dissolution profile of formulations F IV - FVI

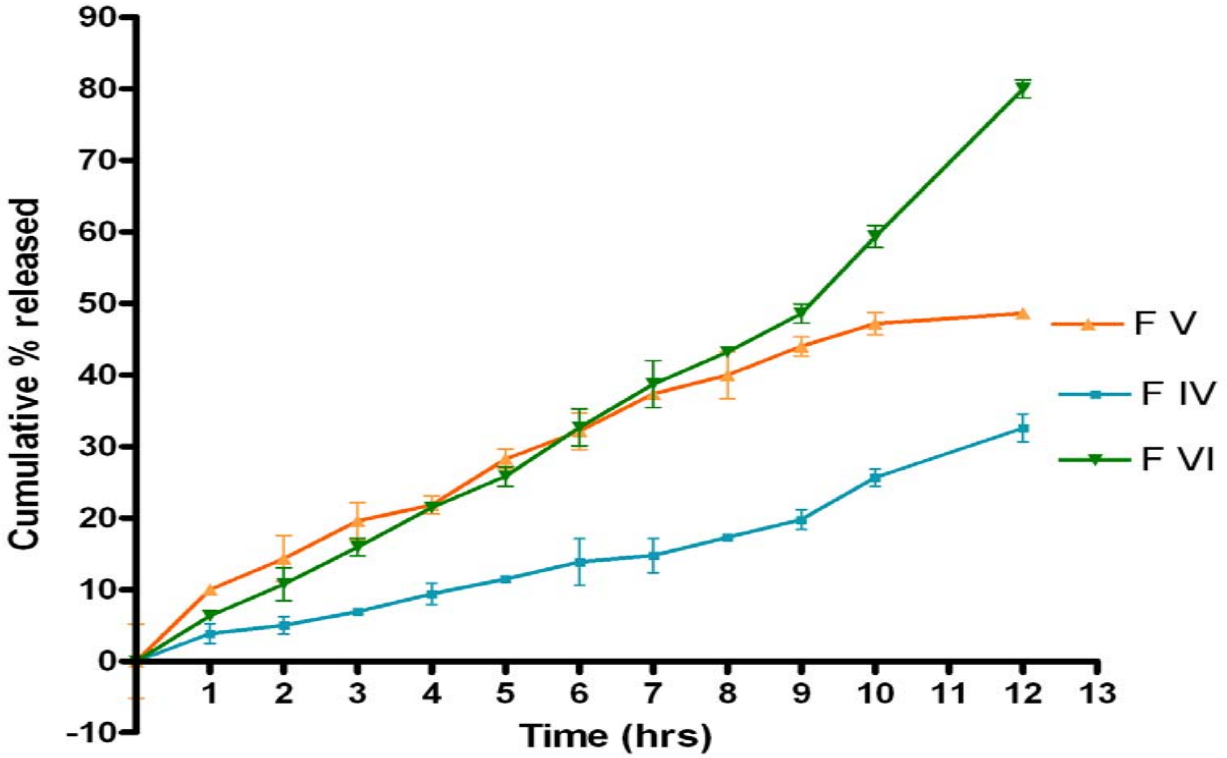


***Table: 6.19 In Vitro* Dissolution Study of 5 FU Floating tablets (F-VII)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	6.0	5.40	5.41 ± 2.36
2	2	7.16	6.45	6.46 ± 0.51
3	3	8.89	8.0	8.02 ± 2.31
4	4	10.31	9.28	9.30 ± 0.64
5	5	12.60	11.34	11.37± 1.54
6	6	14.44	13.0	13.03 ± 0.56
7	7	18.03	16.23	16.27 ± 0.39
8	8	22.44	20.2	20.25 ± 3.62
9	9	26.44	23.80	23.86 ± 1.59
10	10	36.47	32.82	32.90 ± 1.47
11	12	45.08	40.58	40.68 ± 0.38

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.20 In Vitro* Dissolution Study of 5 FU Floating tablets (F-VIII)**

Medium : Simulated Gastric Fluid pH 1. 2, 900 ml
Apparatus : USP basket, Rpm100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	7.30	6.572	6.55± 0.42
2	2	12.0	10.8	10.76± 4.61
3	3	13.47	12.12	12.08± 2.41
4	4	15.66	14.10	13.95± 0.48
5	5	16.98	15.28	15.23 ± 0.57
6	6	18.73	16.86	16.80 ± 3.56
7	7	22.13	19.92	19.85 ± 0.74
8	8	24.37	21.94	21.87 ± 2.51
9	9	35.13	31.62	31.51 ± 1.38
10	10	44.70	40.23	40.09 ± 1.10
11	12	71.42	64.28	64.07 ± 0.47

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.21 In Vitro* Dissolution Study of 5 FU Floating tablets (F-IX)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket , Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	14.59	13.14	13.17 ± 1.03
2	2	20.67	18.60	18.65 ± 1.33
3	3	32.50	29.25	29.34 ± 2.45
4	4	37.99	34.19	34.29 ± 1.04
5	5	40.62	36.56	46.67 ± 2.36
6	6	45.24	40.72	50.84 ± 2.11
7	7	54.27	48.84	58.98 ± 1.31
8	8	62.1	55.89	66.05 ± 2.83
9	9	71.12	64.01	74.20 ± 1.85
10	10	81.17	73.05	83.27± 2.28
11	12	99.38	89.45	94.71 ± 1.47

Cumulative % Drug Release readings are mean ±SE of triplicate values

Release kinetics of FVII

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	2.024167	7.608859957	0.615023179	-0.00498
Correlation	0.975959	0.941179577	0.955024873	-0.99968
r^2	0.952497	0.885818996	0.912072507	0.999359

Release kinetics of FVIII

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	2.001548	7.782827811	0.539472716	-0.00509
Correlation	0.989175	0.986761619	0.990355507	-0.99958
r^2	0.978468	0.973698493	0.98080403	0.999152

Release kinetics of F IX

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	5.817738	22.60481186	0.587532819	-0.0068
Correlation	0.989678	0.98652446	0.991710158	-0.99414
r^2	0.979462	0.97323051	0.983489037	0.988312

Fig: 6.10 *Invitro* dissolution profile of formulations F VII – F IX

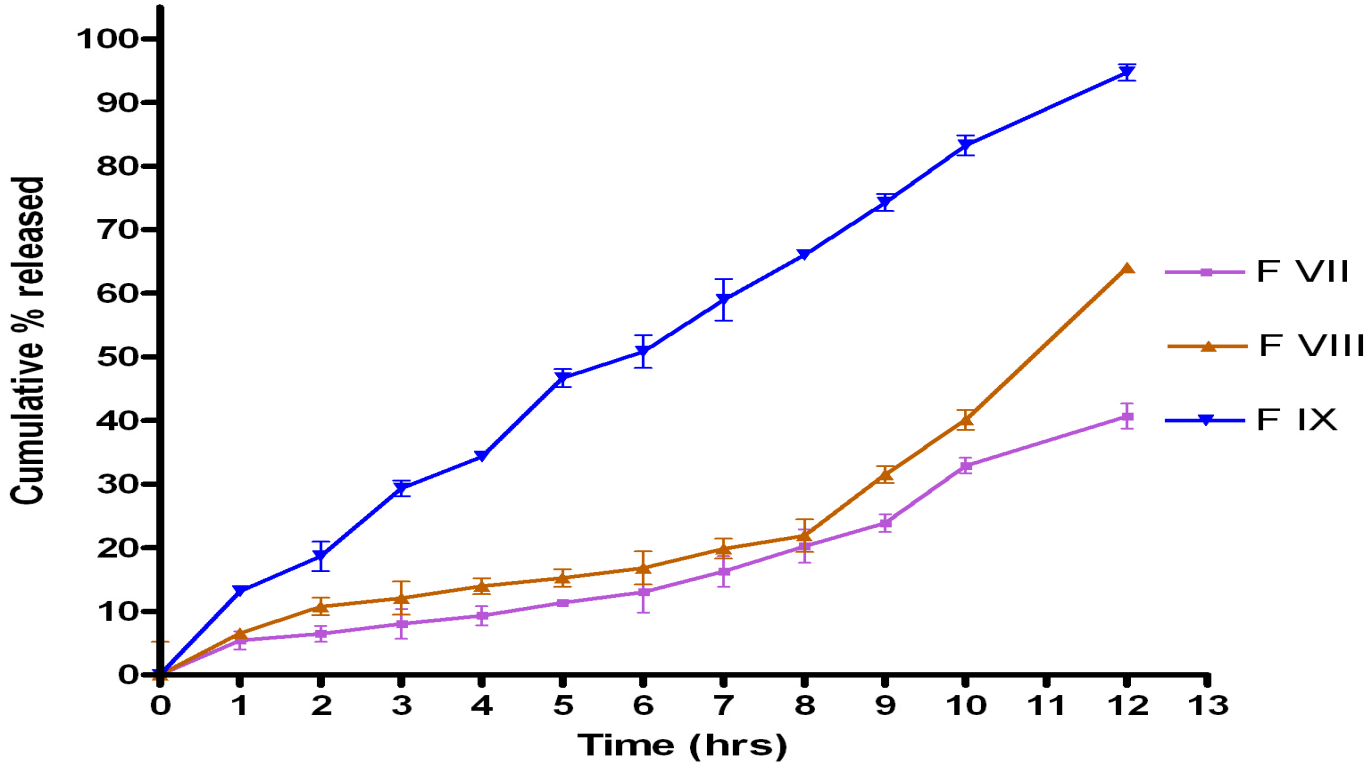


***Table: 6.22 In Vitro* Dissolution Study of 5 FU Floating tablets (F-X)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1.	1	3.07	2.77	2.76 ± 0.37
2.	2	3.98	3.58	3.56 ± 2.38
3	3	5.11	4.60	4.58 ± 2.40
4	4	6.38	5.74	5.71 ± 0.55
5	5	7.83	7.05	7.01 ± 1.31
6	6	9.94	8.95	8.90 ± 2.54
7	7	11.04	9.94	9.89 ± 0.41
8	8	11.83	10.65	10.6 ± 1.34
9	9	13.26	11.93	11.87 ± 0.37
10	10	15.37	13.83	13.76 ± 1.57
11	12	25.58	23.02	22.90 ± 0.32

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.23 In Vitro* Dissolution Study of 5 FU Floating tablets (F-XI)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	5.29	4.764	4.78 ± 1.41
2	2	7.78	7.0	7.03 ± 0.44
3	3	11.23	10.11	10.16 ± 2.66
4	4	14.05	12.65	12.71 ± 2.27
5	5	15.36	13.82	13.88 ± 1.55
6	6	17.16	15.44	15.51 ± 0.23
7	7	17.64	15.88	15.95 ± 1.54
8	8	19.03	17.13	17.21 ± 0.65
9	9	21.71	19.54	19.63 ± 1.44
10	10	24.15	21.74	21.84 ± 1.45
11	12	43.89	39.5	39.70 ± 0.30

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.24 In Vitro* Dissolution Study of 5 FU Floating tablets (F-XII)**

Medium : Simulated Gastric Fluid pH1. 2, 900 ml
Apparatus: USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	5.311	4.78	4.76 ± 0.29
2	2	10.73	9.66	9.62 ± 1.29
3	3	18.05	16.25	16.18 ± 0.45
4	4	24.21	21.79	21.70 ± 2.37
5	5	27.81	25.03	24.91 ± 0.57
6	6	30.91	27.82	27.68 ± 1.30
7	7	36.69	33.03	32.87 ± 0.26
8	8	43.47	39.13	38.94 ± 3.63
9	9	49.51	44.56	44.33 ± 0.52
10	10	51.98	46.79	46.55 ± 1.43
11	12	78.27	70.45	70.10 ± 1.30

Cumulative % Drug Release readings are mean ±SE of triplicate values.

Release kinetics of F X

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	1.199881	4.602572368	0.588575584	-0.00466
Correlation	0.994519	0.978684712	0.981619643	-0.99994
r^2	0.989067	0.957823765	0.963577123	0.999883

Release kinetics of F XI

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	1.771786	7.041827627	0.537782422	-0.00489
Correlation	0.973473	0.992580002	0.992967153	-0.99983
r^2	0.94765	0.985215061	0.985983767	0.999653

Release kinetics of F XII

	First order release	Higuchi release	Korsmeyer peppas release	Zero order release
Slope	4.68119	18.2032732	0.594147501	-0.0056
Correlation	0.99468	0.992301905	0.996225763	-0.99765
r^2	0.989388	0.984663071	0.99246577	0.995313

Fig: 6.11 *Invitro* dissolution profiles of formulations F X – F XII

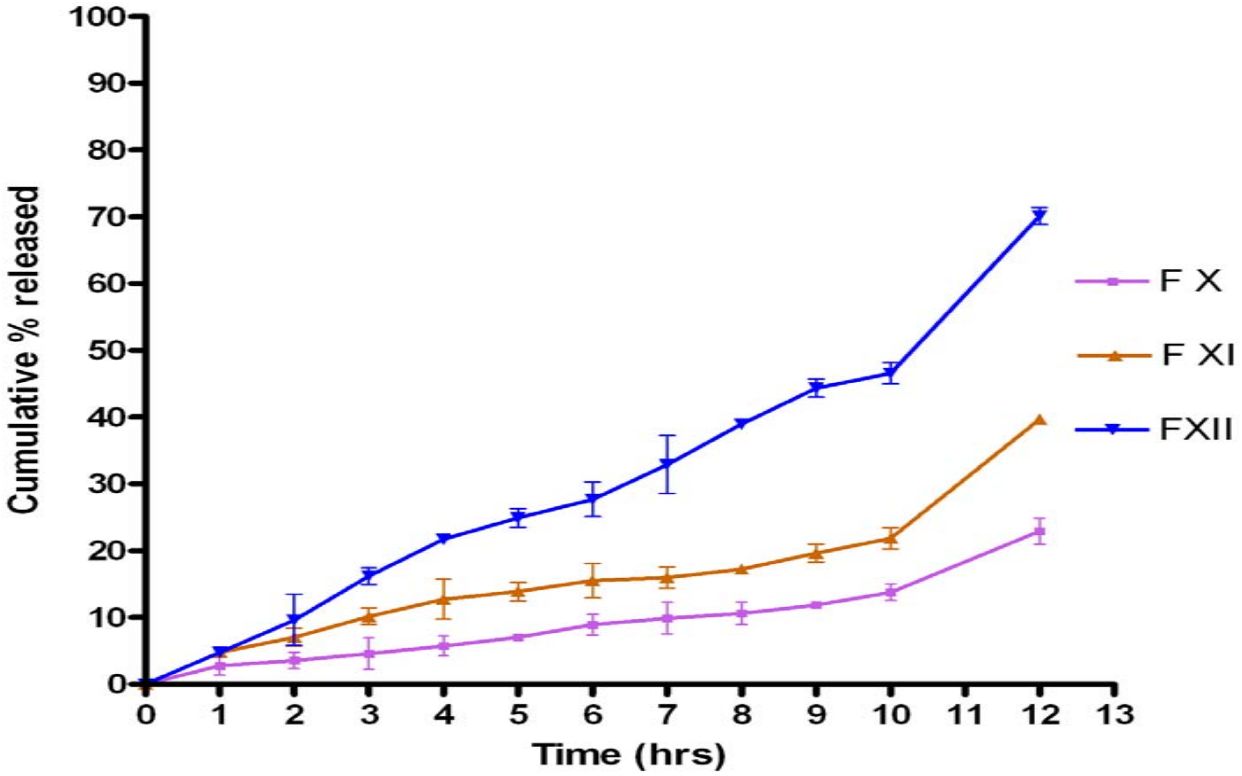


***Table: 6.27 In Vitro* Dissolution Study of 5 FU Floating tablets (F-IXA)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket , Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	14.59	13.14	13.37 ± 3.03
2	2	20.67	18.60	18.65 ± 1.36
3	3	32.50	29.25	29.34 ± 2.44
4	4	37.99	34.19	35.29 ± 2.09
5	5	40.62	36.56	46.67 ± 2.34
6	6	45.24	40.72	50.84 ± 2.17
7	7	54.27	48.84	59.98 ± 1.31
8	8	62.1	55.89	66.05 ± 2.86
9	9	71.12	64.01	74.20 ± 1.84
10	10	81.17	73.05	83.27± 1.28
11	12	99.38	89.45	95.71 ± 2.49

Cumulative % Drug Release readings are mean ±SE of triplicate values

***Table: 6.27a. In Vitro* Dissolution Study of 5 FU Floating tablets (F-IXB)**

Medium : Simulated Gastric Fluid pH 1.2, 900 ml
Apparatus : USP basket , Rpm : 100

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	14.59	13.14	13.19 ± 1.25
2	2	20.67	18.60	19.66 ± 1.65
3	3	32.50	29.25	29.24 ± 2.45
4	4	37.99	34.19	34.69 ± 1.37
5	5	50.62	46.56	46.67 ± 1.36
6	6	65.24	50.72	50.64 ± 2.11
7	7	64.27	58.84	58.98 ± 1.31
8	8	72.1	65.89	66.24 ± 2.83
9	9	81.12	74.01	74.21 ± 1.85
10	10	88.17	83.05	84.28± 1.28
11	12	99.38	94.45	94.91 ± 1.47

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.28 In Vitro* Dissolution Study of 5 FU Floating tablets (F-IXB)**

Medium : Simulated Gastric Fluid pH 1.2
Apparatus: USP basket, Rpm : 50 (fasted state)

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	14.59	13.14	15.17 ± 1.52
2	2	20.67	18.80	22.95 ± 1.36
3	3	32.50	29.95	28.94 ± 2.85
4	4	37.99	34.19	34.26 ± 1.51
5	5	50.62	46.56	45.63 ± 2.48
6	6	65.24	51.72	51.84 ± 1.11
7	7	64.27	58.84	59.18 ± 0.31
8	8	72.1	65.89	66.26 ± 2.85
9	9	81.12	74.01	74.76 ± 1.59
10	10	88.17	83.05	86.27± 3.28
11	12	100.38	95.45	95.06 ± 1.49

Cumulative % Drug Release readings are mean ±SE of triplicate values.

***Table: 6.29 In Vitro* Dissolution Study of 5 FU Floating tablets (F-IXB)**

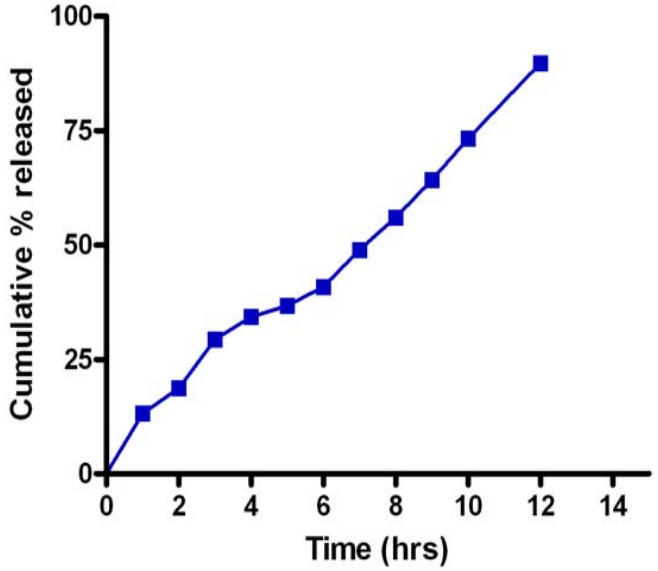
Medium : Simulated Gastric Fluid pH1. 2, 900ml
Apparatus : USP basket , Rpm : 25 (fed state)

S.No	Sampling Time (hrs)	Concentration equivalent to absorbance (mcg/ml)	Cumulative amount of drug released (mg)	Cumulative percentage release ± S.E
1	1	14.59	11.16	14.17 ± 1.37
2	2	20.67	21.19	18.65 ± 1.54
3	3	32.50	23.95	29.34 ± 3.45
4	4	37.99	27.67	34.29 ± 2.04
5	5	50.62	30.26	46.67 ± 2.35
6	6	65.24	33.80	51.84 ± 2.12
7	7	64.27	39.46	58.98 ± 1.34
8	8	72.1	43.36	66.05 ± 2.93
9	9	81.12	46.80	74.20 ± 1.65
10	10	88.17	55.86	86.27± 2.76
11	12	100.38	64.19	94.71 ± 1.64

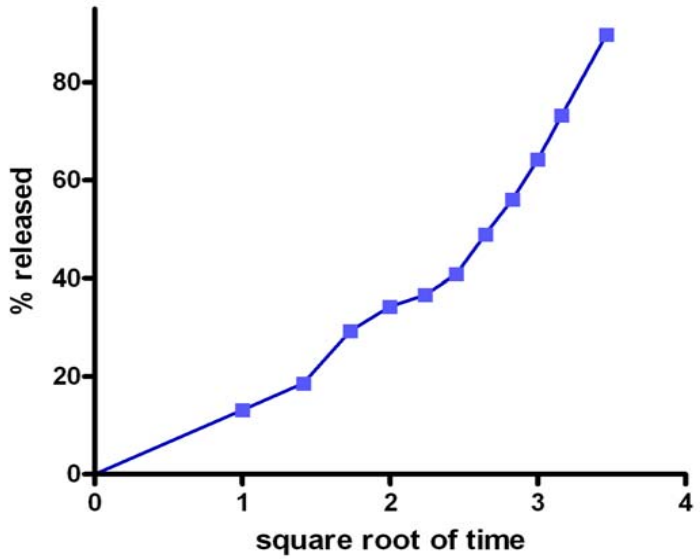
Cumulative % Drug Release readings are mean ±SE of triplicate values.

Fig: 6.15 Release kinetics profile of F IX (Best formulation)

Zero order release



Higuchi release



Korsmeyer Peppas mode of release

First order release

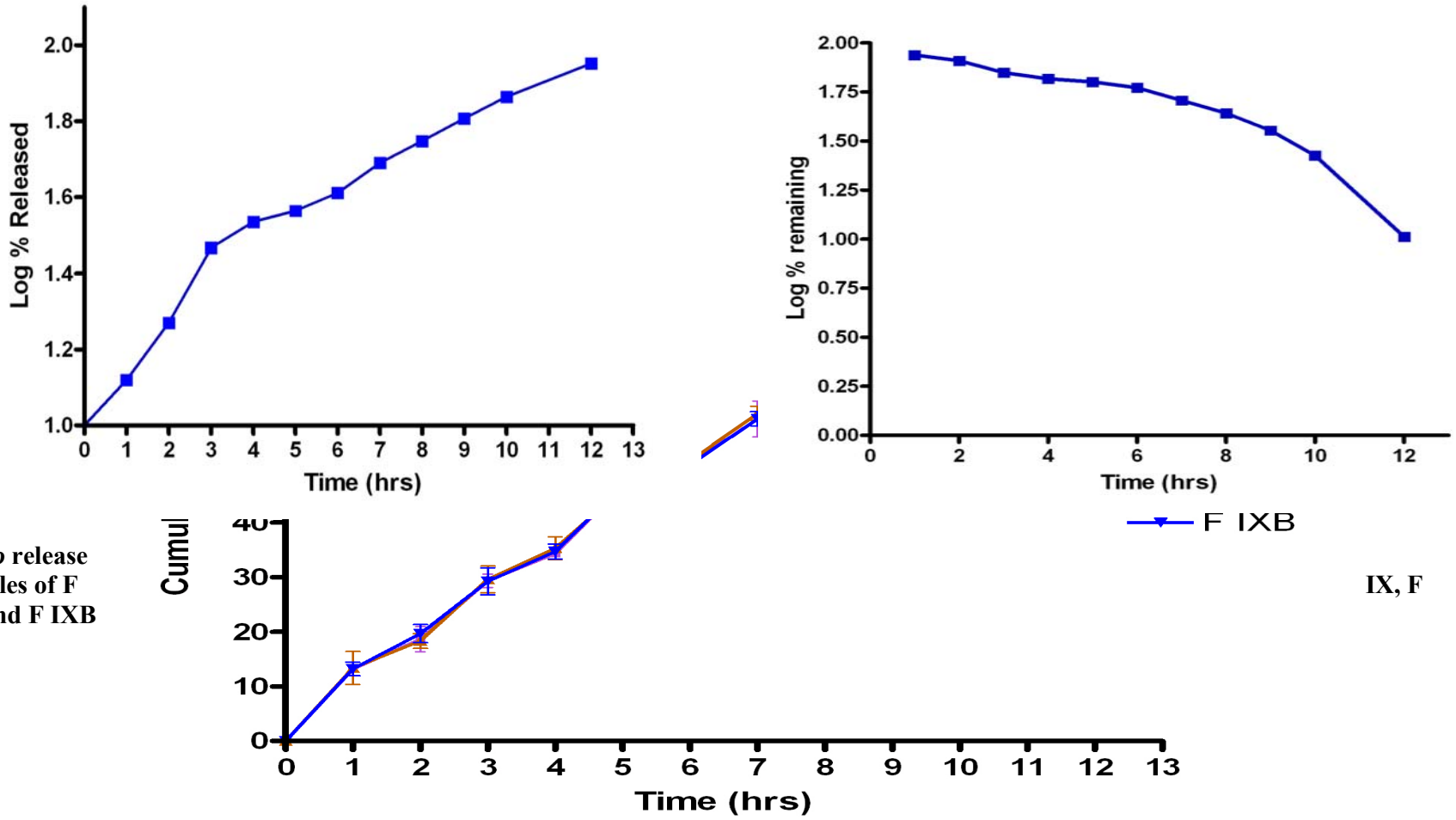


Fig: 6.13 Comparison of *invitro* release profiles of F IXA and F IXB

IX, F

Fig: 6.14 Comparison of *invitro* release profiles of FIXB at 25, 50 and 100 rpm

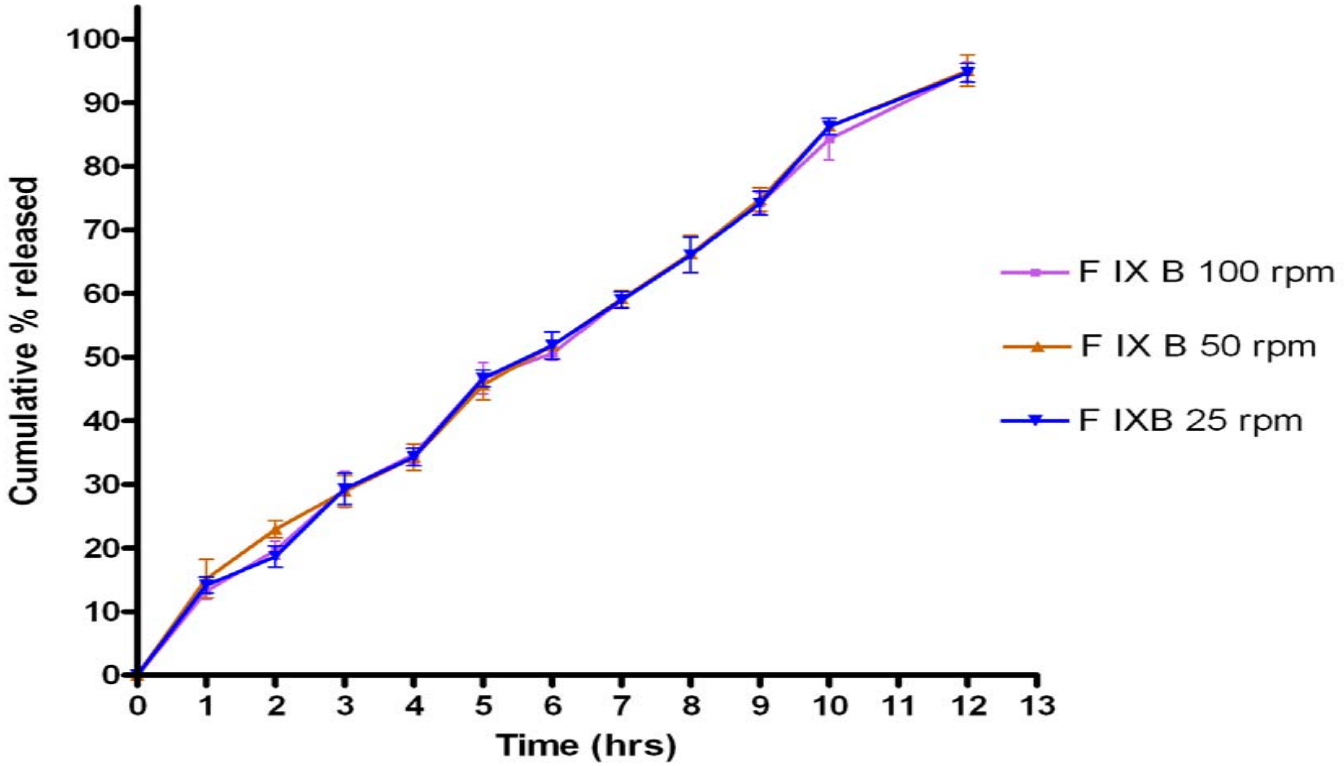

Cumulative % released
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time (hrs)
F IX B 100 rpm
F IX B 50 rpm
F IXB 25 rpm

Table:6.25 **Physical characteristics of Selected 5 FU GRS tablets (F-IXA, F-IXB)**

Batch No	Weight Variation	Friability (%)	Hardness (Kg/cm^2)	Drug Content	Percentage Drug Content
F-IXA	478±0.6	0.029	4.4	99.7 ± 1.04	99.87
F-IXB	475±0.2	0.028	4.4	99. 5 ±1.23	99.84

Table: 6.26 **Buoyancy lag time of Selected 5 FU GRS tablet (F-IXA, F-IXB)**

Batch No	RPM	Buoyancy lag time (Sec)
F- IXA	25	22
	50	21
	100	20
F- IXB	25	26
	50	24
	100	21

Table: 6.26a **Duration of floating of selected 5 FU GRS tablets (F-IXA, F-IXB)**

Batch No	RPM	Duration of Floating (hours)
F- IXA	25	24
	50	24
	100	24
F- IXB	25	24
	50	24
	100	24

Fig: 6.12 Comparison of buoyancy lag time of F IXA and F IXB at different rpm

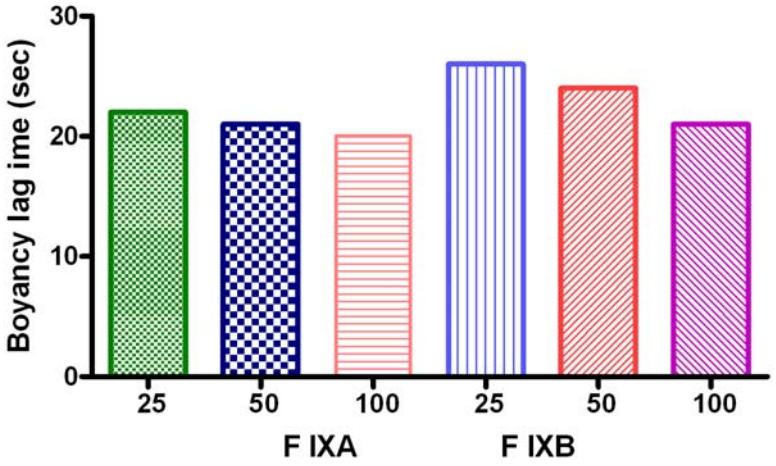


Fig: 6.12a. Comparison of floatation time of F IXA and F IXB at different rpm

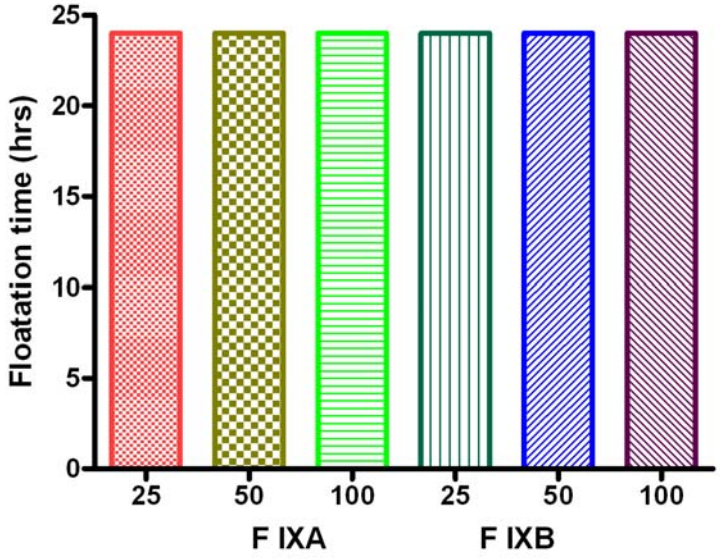


Fig: 6.16 *Invitro* buoyancy study of 5 FU tablets

Fig 6.16a. At 0 seconds

Fig 6.16 b. After 20 seconds

Fig 6.16c. After 24 seconds

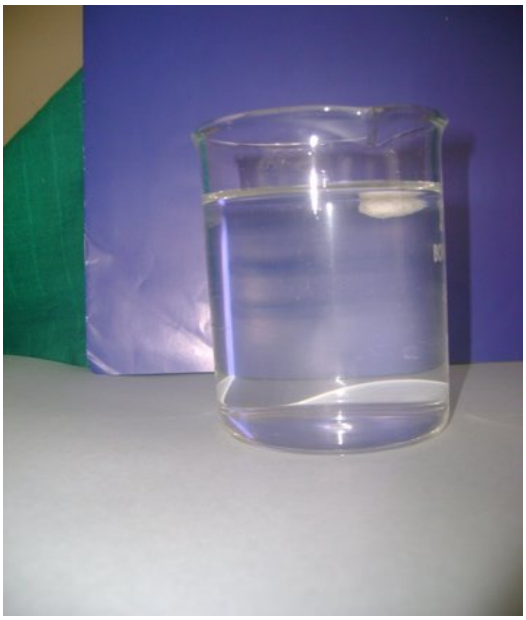


Fig 6.16d. After 12 hours

Fig: 6.17a. SEM photograph of formulation F IXB after 1 hour of dissolution

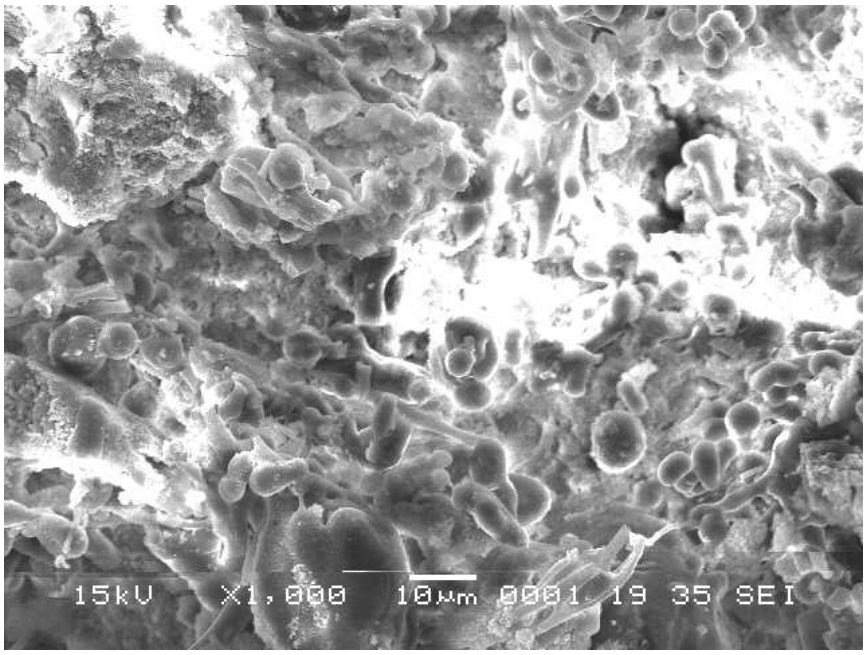
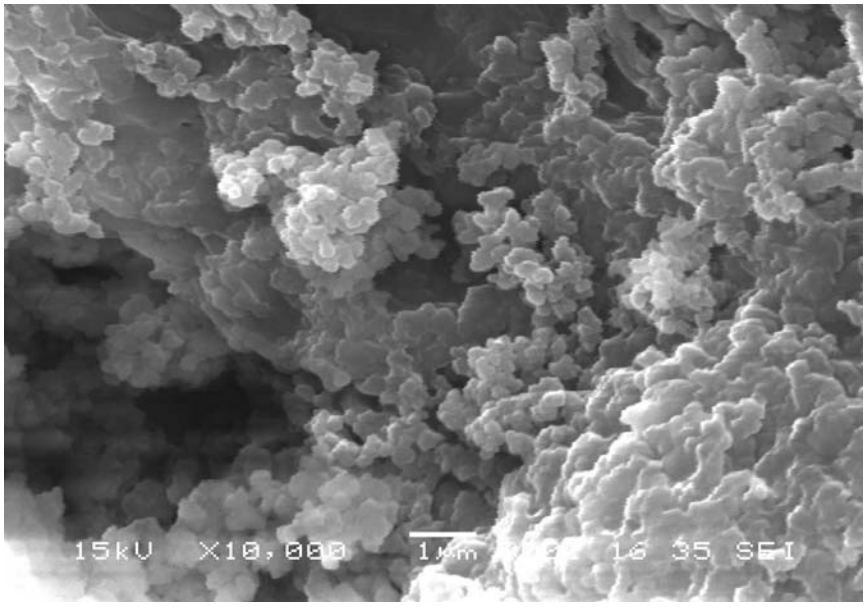


Fig: 6.17b. SEM photograph of formulation F IXB after 12 hour of dissolution



FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF FLUOROURACIL

MATERIALS AND METHODS

EXPERIMENTAL

LIST OF SOLVENTS AND CHEMICALS

Ethanol	SD Fine Chemicals, Mumbai
Dichloromethane	SD Fine Chemicals, Mumbai
Poly vinyl alcohol	Rohm Pharma, Germany
Eudragit RL 100	Rohm Pharma, Germany
Eudragit RS 100	Rohm Pharma, Germany
Eudragit RS PO	Rohm Pharma, Germany
Eudragit RL PO	Rohm Pharma, Germany
Methanol, LR Grade	MERCK, Mumbai
Hydrochloric acid LR Grade	SD Fine Chemicals, Mumbai

LIST OF INSTRUMENTS

Compound Microscope	
Differential Scanning Calorimeter (DSC)	DSC 200 PC Series (Perkin Elmer)
FTIR Spectrophotometer	Boman, model no.MB104, Canada
Humidity Control oven	Sigma instruments, Mumbai
Hot air oven	Minicon, Mumbai
Magnetic Stirrer	Indian Equipment Corporation
Mechanical Stirrer	Remi motors Ltd.Mumbai
Sonicator (Trans-o-Sonic)	Enertech Electronic Pvt.Ltd, Chennai
Tablet Dissolution Test Apparatus	Electrolab TDT-08L, Mumbai
Tablet Hardness Tester	M.C.Dalal Agencies
UV-Visible Spectrophotometer	SHIMADZU UV-1601, Japan
Vacuum Oven	Minicon, Mumbai
Water Bath shaker	Remi Equipment Pvt. Ltd.,India
Weighing Balance	AB104-S, Mettler, Taledo

IR Spectroscopy

The Fourier transformed infrared spectra of drug and polymers were obtained using FTIR Spectrophotometer. This technique is used to determine any chemical interactions between drug and the polymers.

Thermal analysis

Differential scanning calorimetry (DSC) is a rapid analytical technique commonly used for evaluating drug – polymer interactions. DSC of 5FU loaded microspheres was performed with a DSC 200 PC Series (Perkin Elmer Corp). The physical mixture was prepared by simple blending in a mortar and had the same compositions as the loaded microspheres. Samples (5 – 7.5 mg) were scanned in aluminium pans over the temperature range between 50 and 300° C at a scanning rate of 10° K/ min. Nitrogen was used for purging the sample holders at a flow rate of 20 ml/ min to maintain inert atmosphere.

Preparation of microspheres

Microballoons were prepared by the emulsion solvent diffusion method established by Kawashima[74] et al. Drug (5 FU) was dissolved in ethanol (10 ml). Polymers were dissolved or dispersed in a mixture of dichloromethane (10 ml) and water (10ml) at room temperature. The dispersion of polymer was added to the ethanolic solution of drug. The mixture was stirred for 1 hour. To the mixture 30ml of 1% aqueous solution of poly vinyl alcohol was introduced at 40 ºc, forming oil in water type emulsion (o/w). The resultant emulsion was stirred, employing a propeller type agitator at 400 rpm. The finely dispersed droplets of the polymer solution of drug were solidified in the aqueous phase via diffusion of the solvent. After agitating the system for 4 hours, the resulting polymeric particulate systems were filtered and dried in a dessicator for 24 hours.

Four different types of Eudragits with varied characteristics were used to form 5 fluorouracil microspheres. Here, there are three different aspects for the preparation of microspheres.

First, 5 fluorouracil loaded microspheres were prepared by using single polymer, i.e. Eudragit RS100 or Eudragit RSPO or Eudragit RL100 or Eudragit RLPO in the drug-polymer ratio of 1:1 and 1:2 respectively.

Second, 5 fluorouracil loaded microspheres were prepared by using mix polymers, i.e. mixing RS-type with RL-type. The ratio of two polymers RS-type and RL-type was 1:1, while drug to polymer ratio was 1:1 and 1:2 respectively.

Third, the microsphere formulation with better release, when compared to other formulations, was selected as the prototype formulation. The selected formulation was refabricated with HPMC to study their effects on the release pattern.

Based on the results of the studies in chapter V, it was decided to formulate the gastro retentive systems with β cyclodextrin complexed drug, instead of free drug, prepared by freeze drying technique in the ratio of 1:1, which showed a higher dissolution profile when compared to other complexes.

The formula for preparing the formulations from M1 to M18 is shown in table 7.1 and 7.2

Table 7.1: Formulations of 5 FU Microspheres prepared with different Polymers and Polymer mixtures (Drug: Polymer, 1:1)

Contents of Formulations	**M_1**	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**	**M_7**	**M_8**
5 FU(complexed drug) (mg)	300	300	300	300	300	300	300	300
Eudragit RL 100 (mg)	300	-	-	-	150	-	150	-
Eudragit RS 100 (mg)	-	300	-	-	150	-	-	150
Eudragit RLPO (mg)	-	-	300	-	-	150	-	150
Eudragit RSPO (mg)	-	-	-	300	-	150	150	-
Ethanol (ml)	10	10	10	10	10	10	10	10
Dichloromethane(ml)	10	10	10	10	10	10	10	10
Water (ml)	10	10	10	10	10	10	10	10
PVA (1 %aqueous solution)ml	30	30	30	30	30	30	30	30
Drug / Polymer	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**	**1/1**

Table 7.2: Formulations of 5 FU Microspheres prepared with different Polymers and Polymer mixtures (Drug: Polymer, 1:2)

Contents of Formulations	**M_9**	**M_{10}**	**M_{11}**	**M_{12}**	**M_{13}**	**M_{14}**	**M_{15}**	**M_{16}**	**M_{17}**	**M_{18}**
5 FU (complexed drug) (mg)	300	300	300	300	300	300	300	300	300	300
Eudragit RL 100 (mg)	600	-	-	-	300	-	300	-	-	-
Eudragit RS 100 (mg)	-	600	-	-	300	-	-	300	150	150
Eudragit RLPO (mg)	-	-	600	-	-	300	-	300	150	150
Eudragit RSPO (mg)	-	-	-	600	-	300	300	-	-	-
HPMC	-	-	-	-	-	-	-	-	150	150
Ethanol (ml)	10	10	10	10	10	10	10	10	10	10
Dichloromethane (ml)	10	10	10	10	10	10	10	10	10	10
Water (ml)	10	10	10	10	10	10	10	10	10	10
PVA (1%aqueous solution)ml	30	30	30	30	30	30	30	30	30	30
Drug / Polymer	**1/2**	**1/2**	**1/2**	**1/2**	**1/2**	**1/2**	**1/2**	**1/2**	**1/1/0.5**	**1/1/0.5**

HPMC – Hydroxy propyl methyl cellulose

EVALUATION OF MICROSPHERES

Determination of percentage yield value of microspheres

The percentage yield values of microspheres are calculated from the ratio of the total amount of dried and solidified microspheres (A) to the total weight of polymer and drug initially added (B)

$$\text{Percentage yield value} = \left(\frac{A}{B} \right) \times 100$$

Drug loading and drug entrapment efficiency

An accurately weighed sample of microspheres (50 mg) was crushed in a mortar and added to simulated gastric fluid. This mixture was centrifuged at 4000 rpm for 30 minutes, filtered and analyzed spectrophotometrically at 266 nm. The percent drug loading was calculated by dividing the amount of drug in the sample by the weight of microspheres.

% drug content = (weight of drug in microspheres / weight of microspheres recovered) x100

% drug entrapment = (calculated drug concentration/ theoretical drug content) x 100

Particle size and size distribution

To determine the particle size and size distribution, around 200 microspheres from a suspension of microspheres were mounted on a glass slide and their sizes were measured by using an eye piece micrometer in microscope. The eye-piece micrometer was calibrated with the help of a stage micrometer. Average particle size was calculated and results were tabulated and the particle size distribution is represented in figure.

Morphology

The external and internal morphology of the microparticles were studied by scanning electron microscopy (**SEM**). The samples for SEM were prepared by lightly sprinkling the powder on a double adhesive tape stuck to an aluminium stub. The stubs were then coated with gold to a thickness of about 300 Å under an argon atmosphere

using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Jeol JSM – 1600, Tokyo, Japan).

Floating behaviour

Hundred milligrams of the floating microspheres were placed in SGF, (pH 1.2, 100 ml) containing (0.02% w/v) Tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. After 12 hours, the layer of buoyant microspheres was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined[198] by the weight ratio of floating particles to the sum of floating and sinking particles.

Buoyancy (%) = Wf / (Wf + Ws) x 100

Where, Wf and Ws are the weights of the floating and settled microspheres, respectively. All the determinations were made in triplicate.the floating microspheres were shown in photographs (fig)

***In vitro* release study of the microspheres**

Drug release from microspheres was determined by in-vitro dissolution technique using US pharmacopoeia dissolution apparatus II (paddle type). SGF pH 1.2 (900 ml) was used as the dissolution medium. An accurately weighed sample of 5 fluorouracil loaded microspheres was dropped into the dissolution medium maintained at a temperature of 37± 0.5ºc and stirred at a speed of 50 rpm. At different time intervals, 10 ml aliquots of the samples were withdrawn and the volume was replaced with an equivalent amount of fresh dissolution medium. The collected samples were filtered and analyzed at 266 nm using a UV – visible spectrophotometer.

Mathematical model

Data obtained from in-vitro dissolution tests were analysed according to different mathematical model.

Equations[188,189] included are –

a. Zero order

$\%R = Kt$ $t \rightarrow$ time; $K \rightarrow$ Rate constant

b. First order

Log % unreleased = Kt / 2.303

c. Matrix (Higuchi matrix)

$\%R = Kt^{1/2}$ K = Higuchi dissolution constant

d. Peppas – Korsmeyer equation

$\% R = Kt^{n}$

Or Log %R = log K + n log t

Where 'n' value is used to characterize different release mechanisms

After fitting into these models, the selection was based on the comparison of higher determination coefficient (r^2).

RESULTS AND DISCUSSION

IR Spectroscopy (FTIR)

As there was no shifting, deleting and broadening of the peak observed in the spectrum, it can be concluded that no chemical interactions had been occurred, even not H-bonding. . The results are depicted in Fig 11 – 16 of chapter III respectively.

Different scanning calorimetry (DSC)

DSC thermal analysis was done on pure 5 fluorouracil, and the physical mixture of drug and the excipients as that of composition of M_{18} in the ratio of 1:1. The results are depicted in fig 7.1a -7.1d. Thermo grams of the single component(s) and microspheres are shown in figure. The thermogram of pure 5 fluorouracil is having a sharp melting transition of 5FU was observed at 292.1ºC, with T_{peak} 173.73. Hence, greater the crystallinity, the sharper the endotherm. The thermogram of the freeze drying was superimposed with that of corresponding thermogram of formulation. The change in enthalpy as well as T_{peak} ^{o}C of freeze drying was not very significant. The endothermic peak was super imposed. So combining the results of FTIR and DSC of formulation, it could be concluded that the excipients were compatible with that of 5 fluorouracil. On the other hand, broadening of endothermic peaks of formulations indicate that 5 fluorouracil are molecularly dispersed[198] into the polymer compared with that of pure 5 fluorouracil thermogram.

Formation of microspheres

The floating microspheres were prepared by emulsion solvent diffusion technique. A solution of polymer and the drug in ethanol and dichloromethane was poured into an agitated solution of polyvinyl alcohol. The ethanol rapidly partitioned into the external aqueous phase and the polymer precipitated around dichloromethane droplets. The subsequent evaporation of the entrapped dichloromethane led to the formation of internal cavities within the microparticles. It was found that a saturated solution of polymer produced smooth and high yield microspheres. The undissolved polymer produced irregular and rod shaped particles. It is obvious that the rotation speed of the propeller

affets the yield and size distribution of microspheres. As the rotation speed of the propeller increased from 250 to 1000 rpm, the average particle decreased, while maintaining its morphology. The optimum rotation speed for this experimental system was 400 rpm, as judged from the results of particle size and size distribution.

Percentage yield and Entrapment efficiency

5 fluorouracil loaded microspheres having a fairly high yield (71-91%) were obtained with the polymethacrylates with different permeabilities. The results are tabulated in table 7.3 and represented in fig 7.2

The encapsulation efficiencies ranged from 87 – 99.32%. The encapsulation efficiency of the drug depends on the solubility of the drug in the solvent and continuous phase. The incorporation efficiency of formulations, M1 – M8 was more than formulations M9 – M16. The highest incorporation efficiency of formulation having drug : polymer ratio 1:1 can be explained through the fact that the amount of drug per unit polymer is greater than that in other formulations.

It has been stated that efficiencies of the Eudragit RS-type microspheres were higher than those of the Eudragit RL-type microspheres because of the structural differences between polymer types. High content of the ammonium group facilitates the diffusion of a part of entrapped drug to the surrounding medium during preparation. This statement is applicable for the aqueous surrounding medium. This can be proved by encapsulation efficiencies of formulations $M_1 – M_4$ and $M_9 – M_{12}$.

Notably, in the case of RL / RS-type mixture, the encapsulation efficiencies were little higher than those of microspheres prepared by RL100, RS100, RLPO and RSPO individually. However, there was no apparent relationship between encapsulation efficiency and polymer composition. The microspheres of ratio 1:1 exhibited the highest efficiencies, indicating the formation of the most stable emulsion and the most suitable microsphere structures in the RL / RS mixture ratio of 1:1. The results are given in table 7.3 and in fig 7.3 respectively.

Particle size

When the particle sizes were examined, it was seen that the particle size increases with increasing polymer amount. Particle sizes were also observed to be proportional with dispersed phase viscosities. The decrease in the particle size with decreasing polymer amount in the solvent will decrease the viscosity of dispersed phase that causes the decrease in particle size of formulations.

Thus, the mean size was influenced by the content and type of Eudragit used and its ratio in the formulations. The increase in the mean size with increasing polymer concentration was attributed to the fact that higher concentration of polymer in the sample leads to increase in viscosity of the medium which results in fusion of semi-formed particles and producing an overall increase in the size of the microspheres. Notably, Eudragit RL-type microspheres and Eudragit RS-type microspheres made with the same polymer concentration did not show a significant variation in their mean size value. The results are given in table 7.4 – 7.9 and fig 7.4.

Morphology

Eudragit microspheres were predominantly spherical[198] in appearance, however some were found to be elongated. The smooth nature of the microspheres is evident from their SEM photomicrographs. SEM photomicrographs of formulations M5, M6, M7, M8, M9, M11 and M18 are shown in fig 7.5 and 7.6. The hollow nature which is responsible for the floating behavior is evident from the SEM photomicrographs of the formulation (Fig 7.6a).

Percentage buoyancy

The floating test was carried out to investigate the floatability of the prepared microspheres. The floating ability differed according to the formulation tested. The microspheres were spread over the surface of SGF and the fraction of microspheres settled down as a function of time was quantitated. All the formulations showed good floating ability (78.33 ± 8.0 %). More than 75 % of the particles kept floating for at least 10 h. The good buoyancy behavior of the microspheres may be attributed to the hollow nature of the microspheres. Formulation containing eudragit and HPMC showed best

floating ability (85.32 %) in SGF. Tween 20(0.02% w/v), added to SGF, counteracted the downward pulling at the liquid interface by lowering surface tension, because the relatively high surface tension of simulated gastric fluid causes the highest decrease of surface area at the air fluid interface. Floating of microspheres for 10 h was considered satisfactory performance. The results are tabulated in table 7.10 and fig 7.7. The photographs of the microspheres reveal that the floating microspheres floated for more than 10 hours (fig 7.8 and 7.9).

Release profiles

Fig 7.10 to 7.14 and the results tabulated in table 7.11 to7.15 showed that the release of 5 fluorouracil from different formulations having different composition of drug and polymer illustrating the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices which indicated that the release rate was decreased with increasing the amount of polymer. This could be explained by a decreased amount of drug present close to the surface of microspheres containing higher drug polymer ratio and also by the fact that the amount of uncoated drug decreases with higher polymer concentration.

A burst effect of drug release can be observed on the various formulations as shown in Fig. The burst effect can be attributed to the presence of uncovered drug crystals on the surface of the particles. The burst effect of drug release is also depended upon the drug: polymer ratio. From the figure of release profile, it can be observed that burst effect of drug release persists for first 2-3 hours in formulations having drug : polymer ratio 1:1, while in the formulation having drug : polymer ratio 1:2, burst effect can be observed for first 1-2 hours.

From the data given in table 16, it is clear that the release of 5 fluorouracil from Eudragit RL-type was more compared to Eudragit RS-type. This was due to the presence of more functional quaternary ammonium groups (10%) in RL-type than RS-type (5%). It also came on the front that as the amount of polymer in the formulation increased, the drug release decreased.

The dissolution data at different interval indicated that the release of the drug was more in the microspheres prepared by combining Eudragit RS and RL-type. This was due to the fact that inclusion of highly permeable polymer Eudragit RL-type increases the

porosity of the matrix and thus accelerates the drug release, which is attributed to the difference in the content of quaternary ammonium groups.

In most of the cases, a biphasic dissolution profile was observed; the initial rapid drug leakage, and for the remaining time, slow increase in the release or a nearly linear behavior was observed. This slow increase in the release behavior was observed in the formulation M5, M6, M7, M8, M13, M14, M15 and M16 rapid increase in release behavior was observed in the formulation M1, M3, M9 and $M1_1$. Whereas almost a linear behavior after the burst release was observed in the formulation, M2, M4, M10 and $M1_2$. After such a phase, following steps lead to drug release from microparticles into aqueous medium.

1. Formation of pores within the matrix due to the initial drug dissolution from surface.
2. Penetration of the dissolution medium into the microparticles.
3. Dissolution of drug substances inside the microparticles and
4. Drug release by a diffusion process into the aqueous medium.

Hence, under sink conditions, the slowest step described above would be the rate-limiting step for drug release from the microparticles into the aqueous medium.

The cumulative amounts of drug released[199] were tested separately at each time point to see if there were any differences between the formulations. Any time X group interactions between the formulations were determined by analysis of repeated measurements (univariate ANOVA) by using Visual Stat 2005.

Formulations M5, M6, M7, M8			
Hypothesis : Two sided ; Significance level, $\alpha = 0.05$			
Degree of freedom, df = (3.48)			
	F	P value	Fcritical
	0.30	0.82	2.82
Result : F < Fcrit. Hence there is no differences in the release of the drug from microspheres at each interval among M5, M6, M7, M8			
Remarks : This is due to the presence of Eudragit RL and RS-type in the mixture form in all four formulations			

M_9, M_{10} and M_{13}			
Hypothesis : Two sided ; Significance level, $\alpha = 0.05$			
Degree of freedom, df = (1.22)			
	F	P value	Fcritical
	8.93	0.01	4.30
Result : F > Fcritical. Hence there is difference in the release of the drug from above formulations at each time interval			
Remarks : This difference is due to the fact that the release of drug from RS-type is very less (≈ 46%) and RL-type is around 85% in drug polymer ratio of 1:2. So the rate of release will vary from one interval to another. Whereas the release of drug from $M1_3$ is uniform (72.38% upto 12 hrs) as it contains the mixture of two different polymers.			

Co-relation between *invitro* release and particle size

The particle size in mean diameters along with their cumulative percent release is tabulated in table 7.17. Hence from the results, it can be concluded that as the particle size of the microspheres decreased, the release rate of 5 fluorouracil increased. Hence, particle size of microsphere is inversely proportional to the release of drug from microspheres

Statistics of regression and parameters of the mathematical models for the dissolution data of formulations

It can be observed from the table that, for Higuchi model, the determination co-efficient (r^2) of formulations M1 to M16 are significant ($p<0.001$). When compared to higuchi model, the regression coefficient is less for Peppas model, hence the microsphere formulations predominantly involves higuchi diffusion pattern.

Determination coefficient (r^2) of formulations M10, M13, M14, M15, M16 for zero order kinetics, were significant. Similarly, determination coefficient (r^2) of

formulations M2, M4 to M16 for first order kinetics, was also showing the significant results.

Higuchi model is used to study the release of water soluble and low soluble drugs incorporated into solid matrix. The formulations which followed Higuchi model described drug release as a diffusion process based on the Fick's law, square root time dependent.

Peppas model is a simple, semi empirical model, relating exponentially the drug release to the elapsed time. This model is used to analyse the release of pharmaceutical polymeric dosage forms, when the release mechanism is well known or when more than one type of release phenomenon can be involved. The slope, P value and regression coefficient are shown in table 7.18 to 7.20.

The selected formulation with better release (M18) showed a Higuchi diffusion pattern and followed zero order kinetics.

Fig: 7.1 Schematic representation of the preparation of microspheres

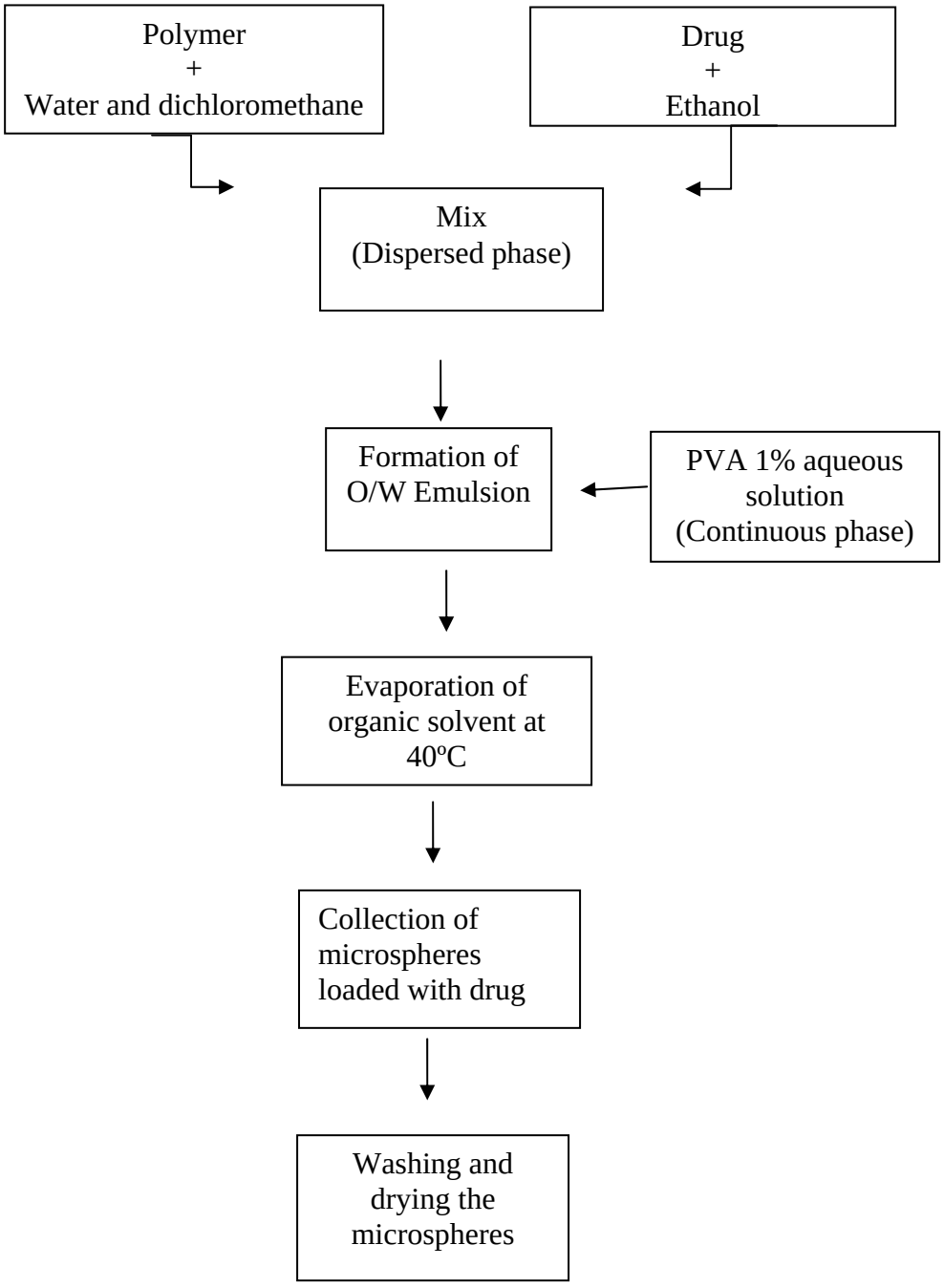


Table 7.3: Percentage yield, drug content and Entrapment of Formulations

Formulation code	Percentage yield (%)	Drug content (%)[a] ± S.E.	Entrapment (%)[a] ± S.E.
M1	61.73	36.52±0.173	90.42±0.427
M2	63.97	35.96±0.11	84.10±0.259
M3	72.63	38.37±0.083	83.96±0.190
M4	65.11	36.46±0.185	84.23±0.428
M5	66.93	40.96±0.038	94.09±0.087
M6	64.73	40.64±0.159	93.34±0.364
M7	55.41	41.06±0.139	94.12±0.321
M8	68.17	40.72±0.189	91.23±0.436
M9	75.09	24.77±0.104	81.7±0.342
$M1_0$	76.13	25.18±0.08	83.01±0.263
M11	77.15	26.79±0.082	88.94±0.270
M12	75.60	27.29±0.026	90.01±0.087
M13	77.41	24.98±0.127	82.50±0.420
M14	80.24	26.63±0.12	87.92±0.397
M15	80.02	26.96±0.121	88.99±0.400
M16	76.43	26.86±0.064	88.88±0.212
M 17	76.58	41.23±0.056	91.11±0.284
M18	78.25	41.36±0.561	91.31±0.265

[a] Mean, n = 3

Fig: 7.2 Comparison of Percentage yield of the formulations

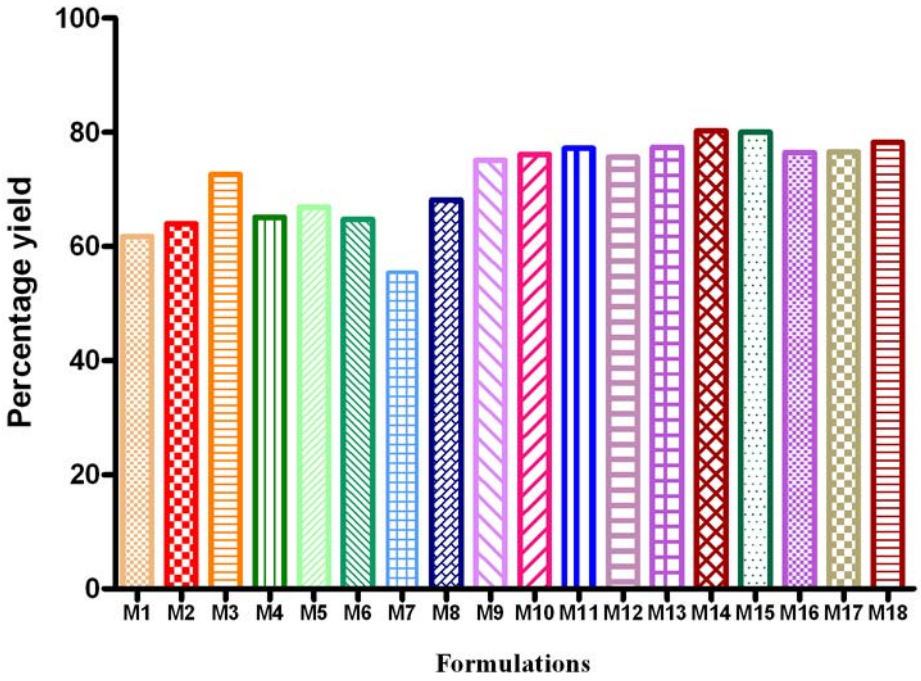


Fig: 7.3 Comparison of entrapment efficiency of the formulations

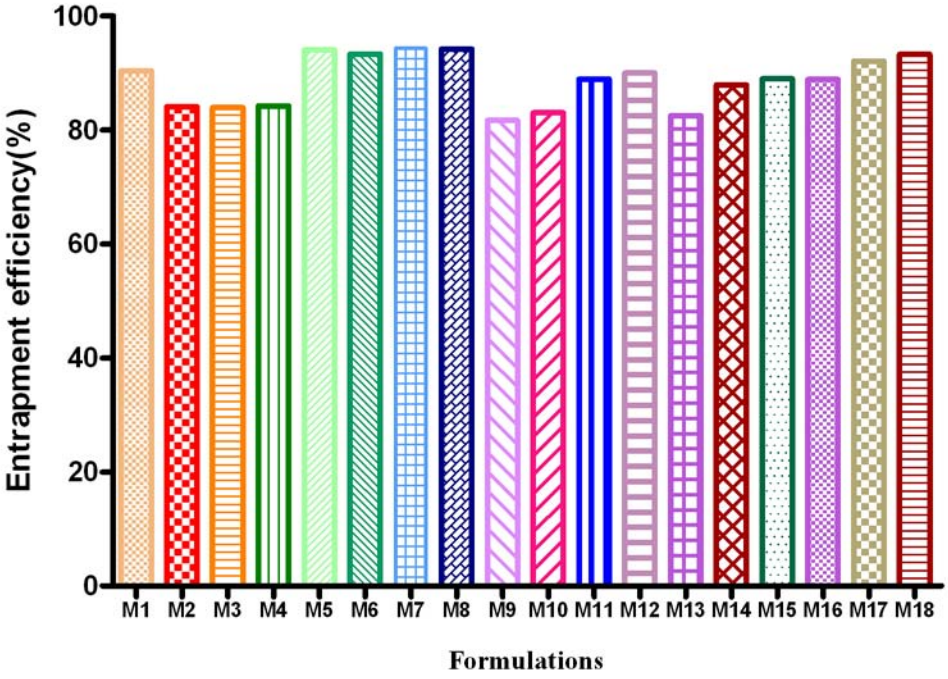


Table: 7.4 Particle size Analysis of Formulation M1 - M4

Particle size Internal (μm)	Mean of size range (μm) d	Frequency of particle in each size range (n) M1	Frequency of particle in each size range (n) M2	Frequency of particle in each size range (n) M3	Frequency of particle in each size range (n) M4
0-50	25	-	-	-	-
50-100	75	-	-	-	-
100-150	125	-	12	30	22
150-200	175	46	54	65	57
200-250	225	60	72	60	70
250-300	275	55	48	15	21
300-350	325	27	8	10	17
350-400	375	8	3	8	9
400-450	425	4	2	7	2
450-500	475	-	1	5	2
		$\sum n = 200$	**$\sum n = 200$**	**$\sum n = 200$**	**$\sum n = 200$**

Table:7. 5 Particle size Analysis of Formulation M5 - M8

Particle size Internal (μm)	Mean of size range (μm) d	Frequency of particle in each size range (n) M5	Frequency of particle in each size range (n) M6	Frequency of particle in each size range (n) M7	Frequency of particle in each size range (n) M8
0-50	25	-	-	-	-
50-100	75	-	2	-	1
100-150	125	-	15	54	52
150-200	175	47	59	59	69
200-250	225	68	74	52	43
250-300	275	49	40	15	26
300-350	325	18	5	15	9
350-400	375	10	2	2	-
400-450	425	6	2	3	-
450-500	475	2	1	-	-
		∑n = 200	**∑n = 200**	**∑n = 200**	**∑n = 200**

Table: 7.6 Particle size Analysis of Formulation M9, M10, M11, M12

Particle size Internal (μm)	Mean of size range (μm) d	Frequency of particle in each size range (n) M9	Frequency of particle in each size range (n) M10	Frequency of particle in each size range (n) M11	Frequency of particle in each size range (n) M12
0-50	25	-	-	-	-
50-100	75	-	-	-	-
100-150	125	-	-	-	3
150-200	175	-	7	5	21
200-250	225	-	44	20	28
250-300	275	9	55	52	23
300-350	325	49	53	57	36
350-400	375	45	24	46	36
400-450	425	53	7	12	21
450-500	475	24	6	2	19
500-550	525	14	2	6	13
550-600	575	6	2	-	-
		∑n = 200	**∑n = 200**	**∑n = 200**	**∑n = 200**

Table:7.7 Particle size Analysis of Formulation M13 M14, M15, M16

Particle size Internal (μm)	Mean of size range (μm) d	Frequency of particle in each size range (n) M13	Frequency of particle in each size range (n) M14	Frequency of particle in each size range (n) M15	Frequency of particle in each size range (n) M16
0-50	25	-	-	-	-
50-100	75	-	-	-	-
100-150	125	-	-	-	-
150-200	175	-	-	-	-
200-250	225	17	8	-	21
250-300	275	64	27	22	46
300-350	325	71	67	39	52
350-400	375	43	54	39	33
400-450	425	5	27	52	25
450-500	475	-	7	22	10
500-550	525		4	12	6
550-600	575		2	6	3
600-650	625		1	4	3
650-700	675		3	4	1
		∑n = 200	**∑n = 200**	**∑n = 200**	**∑n = 200**

Table: 7.8 Particle size Analysis of Formulation $M1_{7,}$ $M1_{8}$

Particle size Internal (μm)	Mean of size range (μm) d	Frequency of particle in each size range (n) M17	Frequency of particle in each size range (n) M18
0-50	25	-	-
50-100	75	1	2
100-150	125	61	49
150-200	175	63	66
200-250	225	42	39
250-300	275	25	29
300-350	325	8	14
350-400	375	-	1
400-450	425	-	-
450-500	475	-	-
		∑n = 200	**∑n = 200**

Table: 7.9 Mean particle size range of different formulations

Formulation	Drug : Polymer ratio	Mean particle size (μm)
M1	1:1	250.75
M2		227.0
M3		221.75
M4		224.75
M5		250.5
M6		218.5
M7		199.0
M8		192.0
M9	1:2	400.0
M10		302.5
M11		323.25
M12		333.25
M13		313.75
M14		359.75
M15		404.25
M16		347.25
M17		191.27
M18		192.35

Fig:7.4 Comparison of mean particle size of the formulations

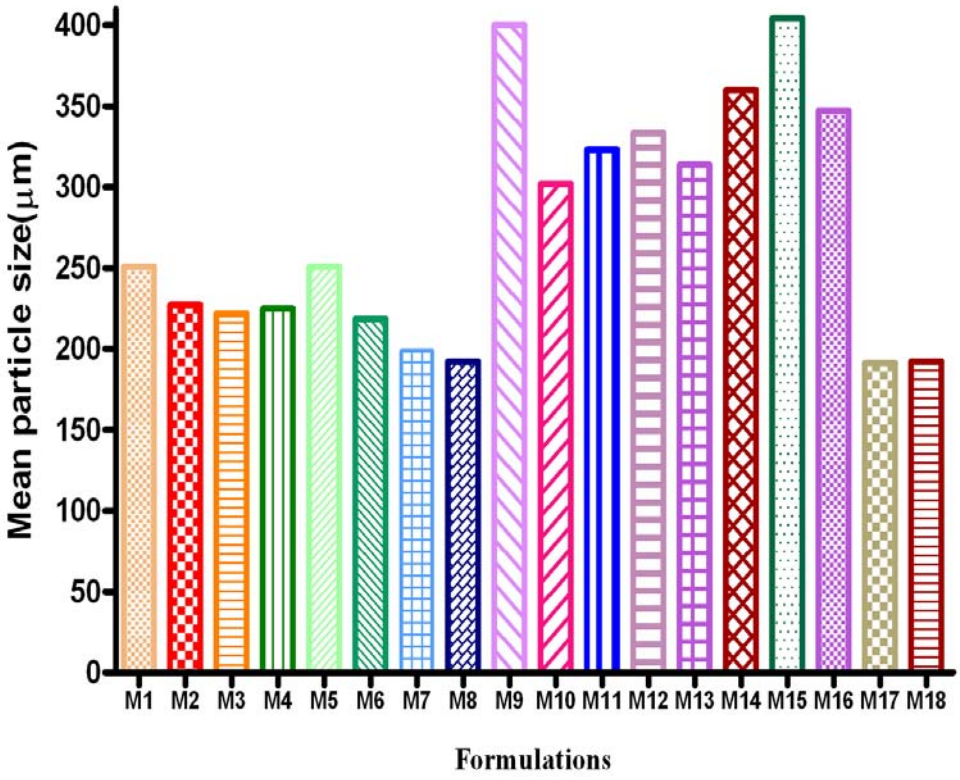


Table: 7.10 Percentage Floating Of Different Formulations of Floating Microspheres

Formulations	**M1**	**M2**	**M3**	**M4**
Buoyancy %	72.1	75.23	73.54	76.24
Formulations	**M5**	**M6**	**M7**	**M8**
Buoyancy %	79.58	81.47	77.32	84.26
Formulations	**M9**	**M10**	**M11**	**M12**
Buoyancy %	69.28	71.96	78.56	74.36
Formulations	**M13**	**M14**	**M15**	**M16**
Buoyancy %	82.65	78.95	83.46	81.47
Formulations	**M17**	**M18**	**-**	**-**
Buoyancy %	85.32	84.23	-	-

Fig: 7.7 Comparison of percentage floating of the formulations

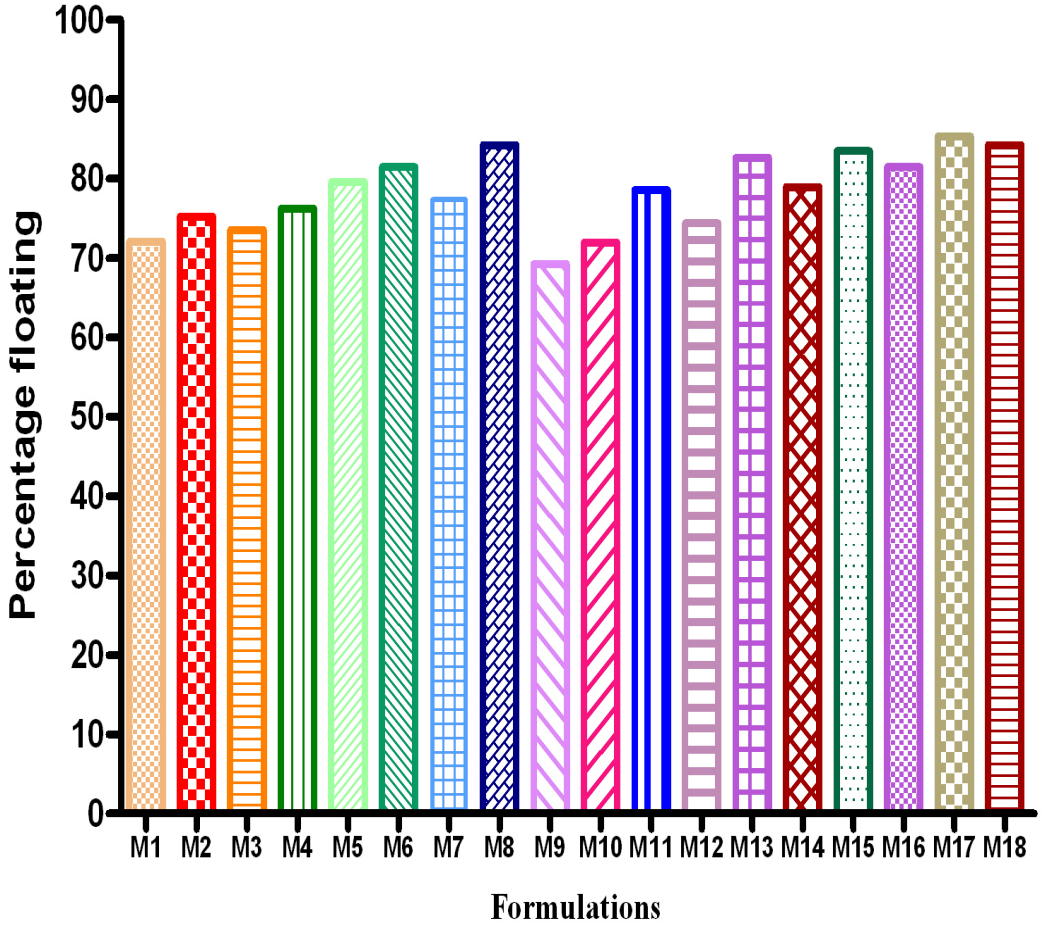


Table: 7.11 Cumulative Percentage Released from 5 Fu Microspheres

Apparatus : USP Dissolution Test Apparatus II
Media: Simulated Gastric Fluid
Type: Paddle
Media Volume: 900 ml
RPM: 100
Temperature: 37±0.5°C

S.NO	TIME(hrs)	CUMULATIVE PERCENT RELEASED*±S.E.			
		Formulation, M1±S.E.	Formulation M2±S.E.	Formulation, M3±S.E.	Formulation M4±S.E.
1	0	0	0	0	0
2	1	38.21± 1.117	15.02± 0.586	39.13± 1.201	15.79± 1.384
3	2	59.45± 1.218	19.90± 1.237	61.76± 2.289	21.23± 2.21
4	3	77.42± 2.490	26.92± 2.355	77.01± 0.180	33.16± 2.356
5	4	79.09± 1.376	31.61± 1.406	78.89± 4.141	39.06± 1.544
6	5	83.83± 0.991	34.96± 1.355	82.59± 0.308	41.88± 2.815
7	6	86 ± 2.484	37.84± 2.551	85.82± 2.117	43.74± 0.342
8	7	90.60± 1.332	41.15± 3.333	90.37± 1.092	47.88± 1.476
9	8	96.47± 3.491	42.95± 0.442	94.72± 0.138	50.02± 2.486
10	9	98.54± 0.402	45.57± 1.204	99.24± 3.361	53.72± 1.377
11	10	99.72± 2.204	48.96± 0.747	----------	55.38± 0.797
12	12	--------	53.47± 0.433	----------	59.63± 3.322

*n = no of trials for each formulation = 3

Table: 7.12 Cumulative Percentage Released from 5 Fu Microspheres

Apparatus : USP Dissolution Test Apparatus II
Media: Simulated Gastric Fluid
Type: Paddle
Media Volume: 900 ml
RPM: 100
Temperature: 37±0.5°C

SL.NO.	TIME(hrs)	CUMULATIVE PERCENT RELEASED*±S.E.			
		Formulation, M5±S.E.	Formulation M6±S.E.	Formulation, M7±S.E.	Formulation M8±S.E.
1	0	0	0	0	0
2	1	15.25± 1.176	19.26± 1.227	21.49± 1.193	23.17± 1.088
3	2	32.32± 2.261	39.56± 0.386	42.10± 2.096	45.25± 1.217
4	3	43.39± 3.351	51.98± 2.434	50.18± 0.198	60.35± 1.082
5	4	50.65± 0.180	59.62± 0.333	54.20± 1.177	68.97± 2.355
6	5	59.28± 2.929	68.23± 1.917	60.16± 0.429	72.28± 0.706
7	6	68.59± 0.603	76.08± 2.540	70.01± 2.235	78.74± 1.344
8	7	76.28± 1.459	78.71± 2.459	74.94± 0.128	81.68± 0.098
9	8	80.72± 0.364	84.14± 0.294	81.70± 1.229	84.28± 3.384
10	9	83.16± 2.500	86.96± 1.280	84.68± 0.067	88.67± 0.191
11	10	88.53± 0.854	90.15± 1.723	87.90± 2.289	93.29± 1.668
12	12	90.69± 1.474	91.29± 0.322	89.49± 3.0829	97.09± 0.378

*n=No. of trials for each formulation=3

Fig: 7.10 Comparison of release profiles of formulations M1 - M4

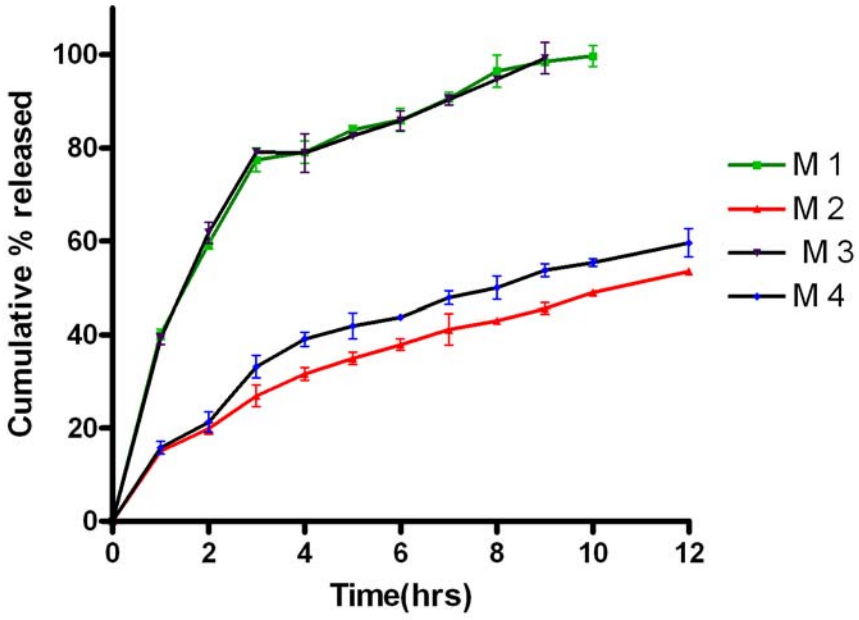


Fig: 7.11 Comparison of release profiles of formulations M5 - M8

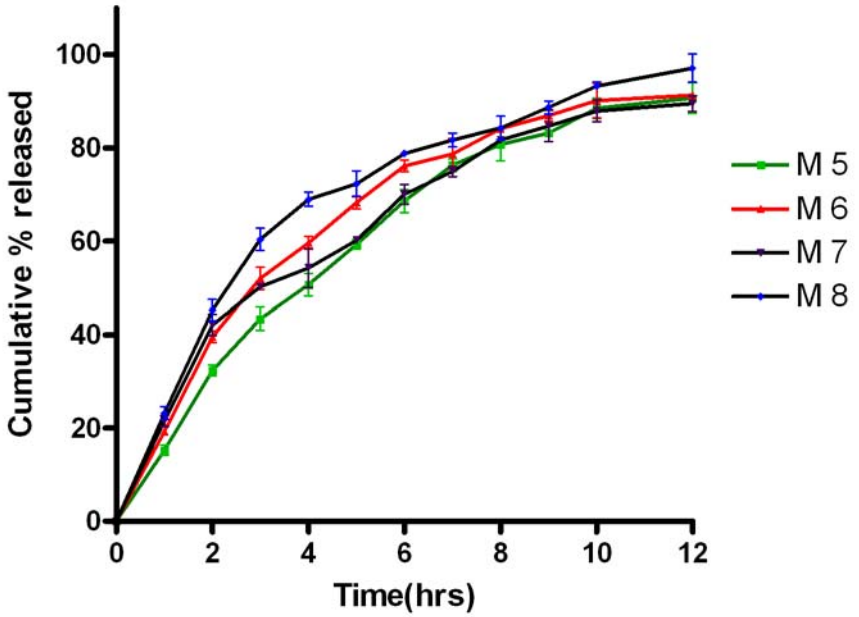


Table: 7.13 Cumulative Percentage Released from 5 Fu Microspheres

Apparatus : USP Dissolution Test Apparatus II
Media: Simulated Gastric Fluid
Type: Paddle
Media Volume: 900 ml
RPM: 100
Temperature: 37±0.5°C

SL.NO.	TIME(hrs)	CUMULATIVE PERCENT RELEASED* ±S.E.			
		Formulation M9±S.E.	Formulation M10±S.E.	Formulation M11±S.E.	Formulation M12±S.E.
1	0	0	0	0	0
2	1	17.48± 0.301	14.44± 1.819	32.54± 3.489	13.69± 1.371
3	2	38.37± 0.963	15.79± 0.477	52.03± 0.797	15.10± 1.168
4	3	44.26± 1.107	19.70± 3.512	61.33± 0.986	19.90± 0.383
5	4	49.91± 1.102	21.52± 0.657	64.21± 1.107	20.63± 0.141
6	5	55.75± 2.573	23.38± 1.631	72.69± 2.266	25.26± 0.435
7	6	61.47± 1.360	28.16± 1.722	75.99± 1.192	27.33± 3.195
8	7	67.38± 1.147	32.27± 0.648	80.68± 1.099	31.11± 3.140
9	8	75.45± 1.043	35.28± 0.895	84.61± 1.072	32.53± 0.233
10	9	77.19± 1.158	37.83± 4.773	86.18± 0.983	36.56± 0.193
11	10	81.17± 2.095	40.62± 1.919	90.06± 1.88	38.82± 0.407
12	12	89.14± 1.136	46.40± 1.019	96.23± 1.258	45.53± 2.450

*n=No.of trials for each formulation=3

Table 7.14 Cumulative Percentage Released from 5 fu Microspheres

Apparatus : USP Dissolution Test Apparatus II
Media: Simulated Gastric Fluid
Type: Paddle
Media Volume: 900 ml
RPM: 100
Temperature: 37±0.5°C

SL.NO.	TIME(hrs)	CUMULATIVE PERCENT RELEASED* ±S.E.			
		Formulation M13±S.E.	Formulation M14±S.E.	Formulation M15±S.E.	Formulation M16±S.E.
1	0	0	0	0	0
2	1	10.36± 0.327	8.59± 0.454	7.39 ± 0.176	9.36± 0.176
3	2	15.19± 0.300	13.72± 0.165	12.46± 0.232	14.44± 0.272
4	3	21.11± 0.264	19.06± 0.196	17.87± 0.331	19.93± 0.130
5	4	24.06± 0.403	22.33± 0.405	20.52± 0.393	24.84± 0.071
6	5	28.62± 0.622	25.86± 0.761	26.08± 1.032	29.98± 0.228
7	6	32.30± 0.277	30.80± 0.354	32.73± 0.475	35.05± 0.104
8	7	37.69± 0.291	38.87± 0.268	35.33± 0.477	40.31± 0.134
9	8	46.58± 0.343	42.14± 0.333	39.65± 0.391	44.33± 0.131
10	9	50.73± 0.388	48.22± 0.185	45.74± 0.425	48.53± 0.127
11	10	55.40± 0.804	52.60± 0.614	50.86± 0.659	55.29± 0.186
12	12	72.39± 0.376	69.37± 0.292	65.23± 0.158	68.48± 0.431

*n=No.of trials for each formulation=3

Table: 7.15 Cumulative Percentage Released from 5 fu Microspheres

Apparatus : USP Dissolution Test Apparatus II
Media: Simulated Gastric Fluid
Type: Paddle
Media Volume: 900 ml
RPM: 100
Temperature: 37±0.5°C

Sl.No	Time (hrs)	Cumulative % Released M17	Cumulative % released M18
1	0	0	0
2	1	22.78± 0.088	24.98 ± 0.453
3	2	45.25± 0.217	35.79 ± 0.344
4	3	56.35± 0.082	49.23 ± 0.176
5	4	58.34± 0.355	59.45 ± 0.229
6	5	65.28± 0.706	66.34 ± 0.622
7	6	70.74± 0.344	70.90 ± 0.761
8	7	76.68± 0.098	78.95 ± 0.327
9	8	78.96± 0.384	81.90 ± 0.425
10	9	82.67± 0.191	87.78 ± 0.098
11	10	90.29± 0.668	93.87 ± 0.130
12	12	96.12± 0.230	97.99 ± 0.191

*n=No.of trials for each formulation=3

Fig:7.12Comparison of release profiles of formulations M9 – M12

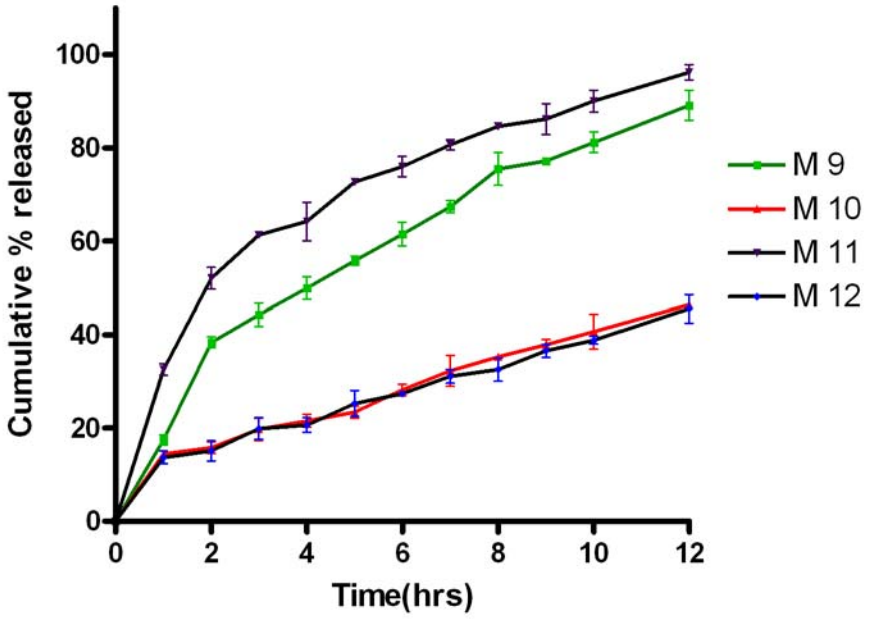


Fig: 7.13 Comparison of release profiles of formulations M13 - M16

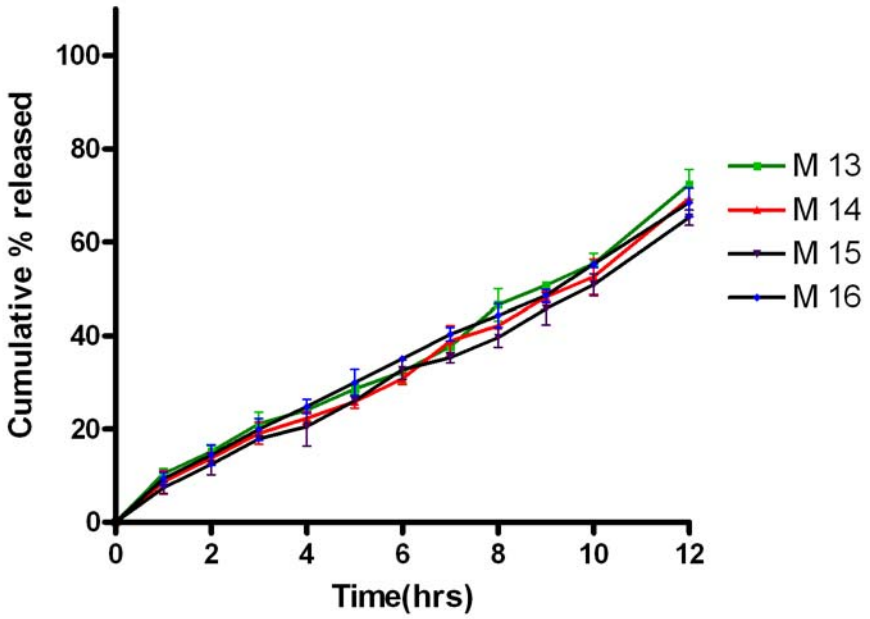


Fig: 7.14 Comparison of release profiles of formulations M8, M17 and M18

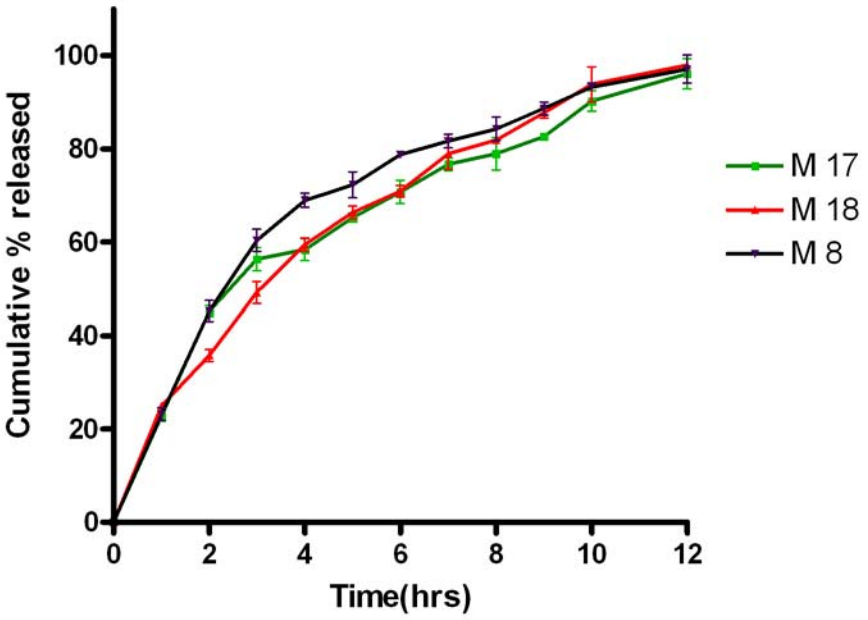


Table 7.16: Cumulative Percent Release of 5 Fluorouracil from Microspheres at Different Time Intervals

Formulation	Composition	Drug : Polymer ratio	Release of 5 FU at			
			1 hr (%)	4 hrs (%)	8 hrs (%)	12 hrs (%)
M1	RL100	1 : 1	38.21	79.09	96.47	100
M2	RS100		15.02	31.61	42.95	58.03
M3	RLPO		39.13	78.89	94.72	100
M4	RSPO		15.79	33.16	50.02	63.99
M5	RL100:RS100		15.25	50.65	80.72	91.08
M6	RLPO:RSPO		19.26	59.62	84.14	92.95
M7	RL100:RSPO		21.49	54.20	81.70	91.70
M8	RS100:RLPO		23.17	68.97	84.28	97.16
M9	RL100	1 : 2	17.48	44.26	67.38	84.59
M10	RS100		14.44	21.52	35.28	46.40
M11	RLPO		32.54	64.21	84.61	93.68
M12	RSPO		13.69	20.63	32.53	45.53
M13	RL100:RS100		10.36	24.06	46.58	72.39
M14	RLPO:RSPO		8.59	22.33	42.14	69.37
M15	RL100:RSPO		7.39	20.52	39.65	65.23
M16	RS100:RLPO		9.36	19.93	44.33	68.48
M17	RS100:RLPO: HPMC	1: 1 :1	22.78	58.34	78.96	96.12
M18	RS100:RLPO: HPMC	1: 1 :1	24.98	59.45	81.90	97.99

Table: 7.17 Co-Relation between *InVitro* Release and Particle Size

Formulation	Drug Polymer ratio	Particle size (μm)	Cumulative % released at 12 hrs
M5	↓	250.5	91.08
M6		218.5	92.95
M7	1:1	199	91.70
M8	↑	192	97.16
M13	↓	313.75	72.39
M14	1:2	359.75	69.37
M15	↑	404.25	65.23
M16		347.25	68.48
M17	1:1	191.27	96.12
M18	1:1	189.25	97.99

Table 7.18: **Descriptive Statistics of Regression and Parameters of the Mathematical Models for the Dissolution Data of Formulations* (Part-I)**

Model	Statistics	M1	M2	M3	M4	M5	M6	M7	M8
First order	r^2 P Slope	0.388 >0.1 12.81	0.81 <0.01 5.353	0.431 >0.1 13.75	0.76 <0.01 6.13	0.852 <0.001 7.12	0.718 <0.01 9.791	0.746 <0.01 9.452	0.572 <0.01 10.11
Zero order	r^2 P Slope	0.855 <0.01 0.523	0.976 <0.001 0.027	0.804 <0.01 0.04	0.97 <0.001 0.074	0.99 <0.001 0.223	0.994 <0.00 0.24	0.986 <0.001 0.219	0.968 <0.001 0.263
Higuchi	r^2 P Slope	0.927 <0.001 35.22	0.991 <0.001 15.69	0.933 <0.001 35.93	0.989 <0.001 18.02	0.976 <0.001 27.23	0.982 <0.001 28.89	0.991 <0.001 27.84	0.965 <0.001 30.06
Peppas	r^2 P slope	0.933 <0.001 43.45	0.993 <0.001 14.62	0.953 <0.001 44.16	0.977 <0.001 16.26	0.974 <0.001 18.24	0.952 <0.00 23.88	0.971 <0.001 25	0.929 <0.001 29.17

P=Critical values for the correlation coefficient
*n=Number of readings=12

Table 7.19: **Descriptive Statistics of Regression and Parameters of the Mathematical Models for the Dissolution Data of Formulations * (Part-II)**

Model	Statistics	M9	M10	M11	M12	M13	M14	M15	M16
First order	r^2 P Slope	0.76 <0.01 8.584	0.869 <0.001 4.255	0.433 >0.1 9.962	0.858 <0.001 4.119	0.985 <0.001 5.75	0.988 <0.001 5.436	0.992 <0.001 5.199	0.988 <0.001 5.634
Zero order	r^2 P Slope	0.991 <0.001 0.154	0.98 <0.001 0.046	0.975 <0.001 0.226	0.979 <0.001 0.044	0.935 <0.001 0.092	0.94 <0.001 0.085	0.954 <0.001 0.081	0.967 <0.001 0.085
Higuchi	r^2 P Slope	0.988 <0.001 1.329	0.969 <0.001 1.076	0.954 <0.01 1.563	0.975 <0.001 1.064	0.871 <0.001 0.956	0.864 <0.01 0.888	0.868 <0.001 0.837	0.899 <0.001 0.933
Peppas	r^2 P slope	0.959 <0.001 21.33	0.941 <0.001 11.91	0.97 <0.001 36.56	0.956 <0.001 11.59	0.977 <0.001 9.036	0.985 <0.001 7.73	0.992 <0.001 6.87	0.994 <0.001 8.57

P=Critical values for the correlation coefficient

*n=Number of readings=12

Table 7.20: Descriptive Statistics of Regression and Parameters of the Mathematical Models for the Dissolution Data of Formulations * (Part-III)

Model	Statistics	M17	M18
Zero order	r^2 P Slope	0.915917 <0.001 7.1490	0.963943 <0.001 8.16833
First order	r^2 P Slope	0.761277 <0.01 0.062731	0.889563 <0.01 0.069650
Higuchi	r^2 P Slope	0.956804 <0.001 28.79	0.993213 <0.001 32.31
Peppas	r^2 P slope	0.938842 <0.001 0.558598	0.9932011 <0.001 0.590132

P=Critical values for the correlation coefficient

*n=Number of readings=12

Fig: 7.6 SEM Photomicrograph of spherical microspheres (M 18)

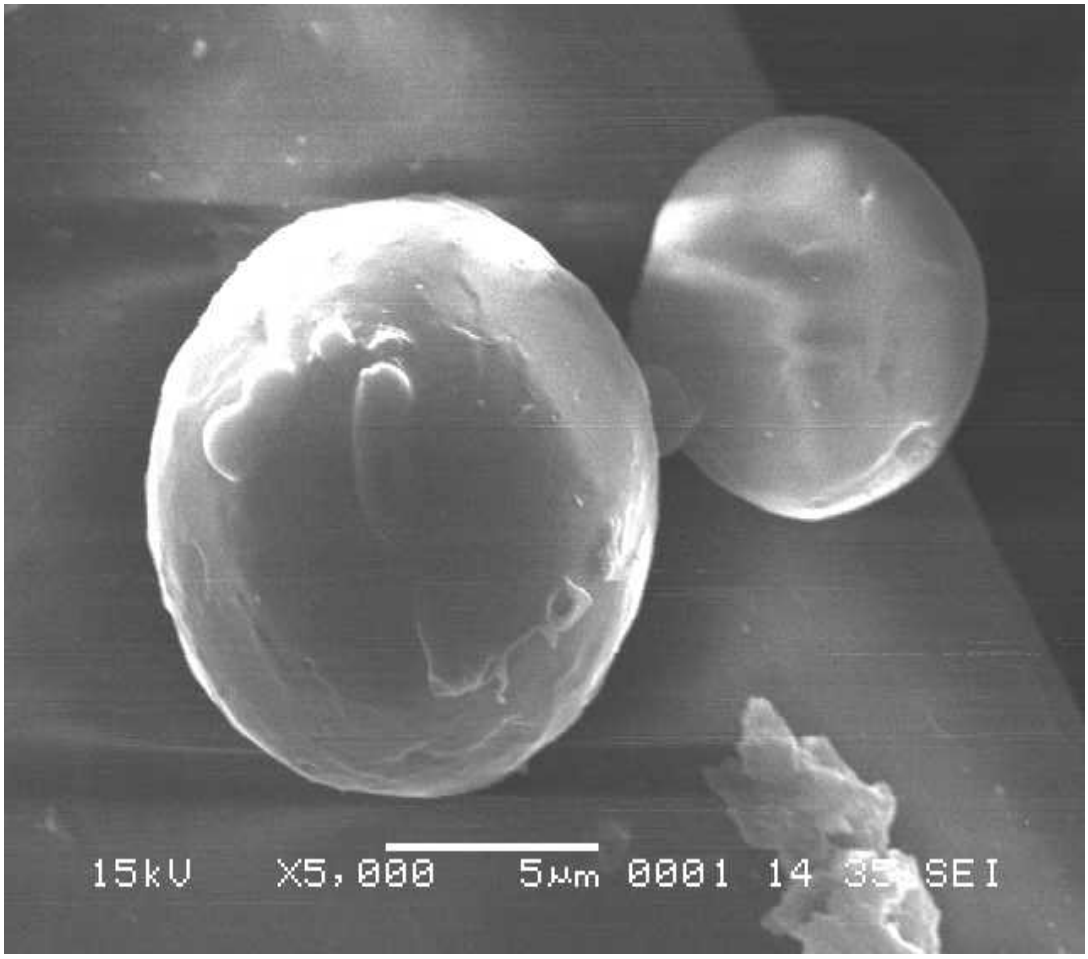


Fig: 7.8 Photograph showing the microspheres rising to the surface (1 min)

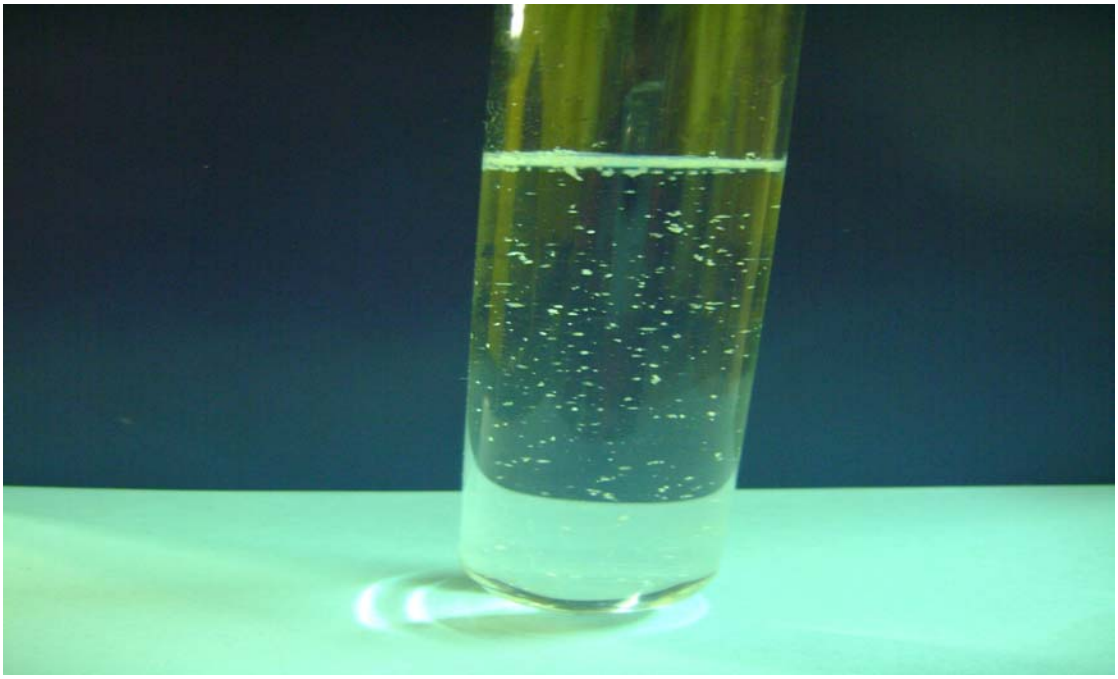


Fig: 7.9 Photograph showing the floating microspheres after 12 hours

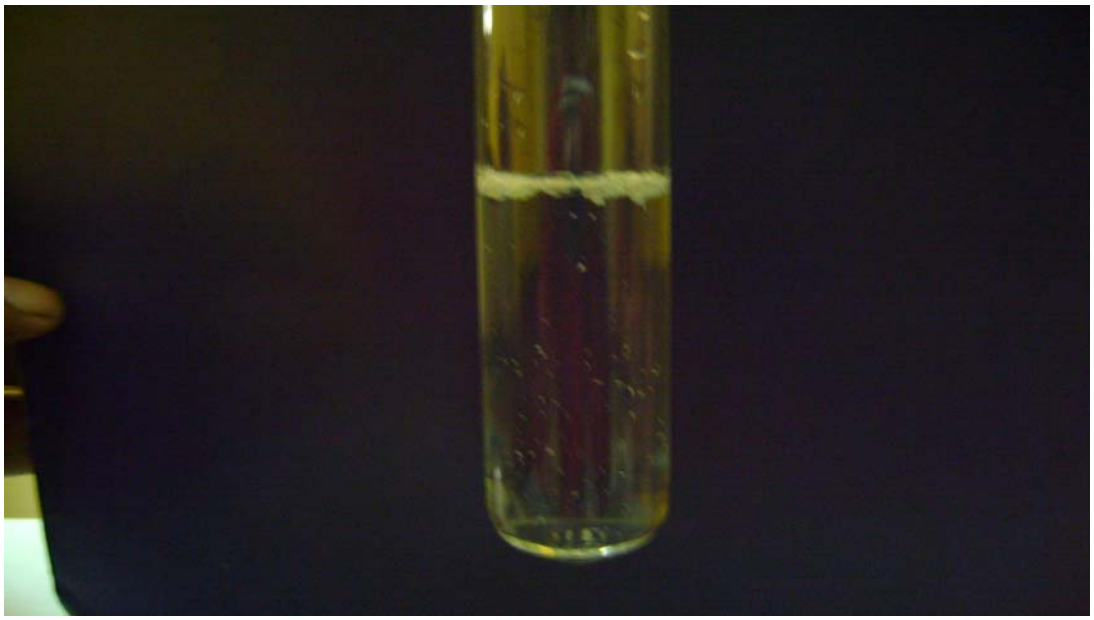


Fig 7.1a: DSC thermogram of Fluorouracil

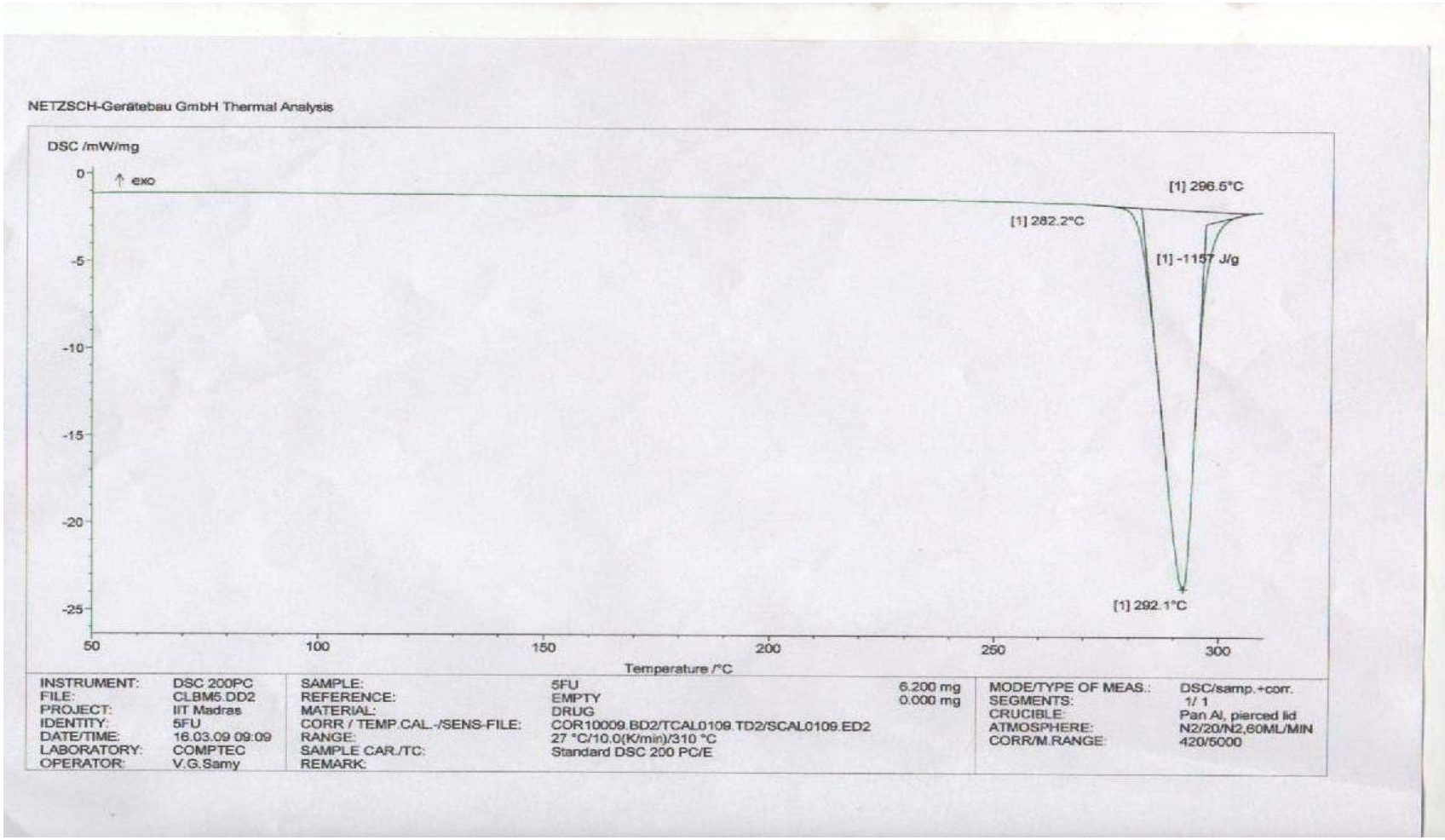


Fig 7.1b: DSC thermogram of β Cyclodextrin

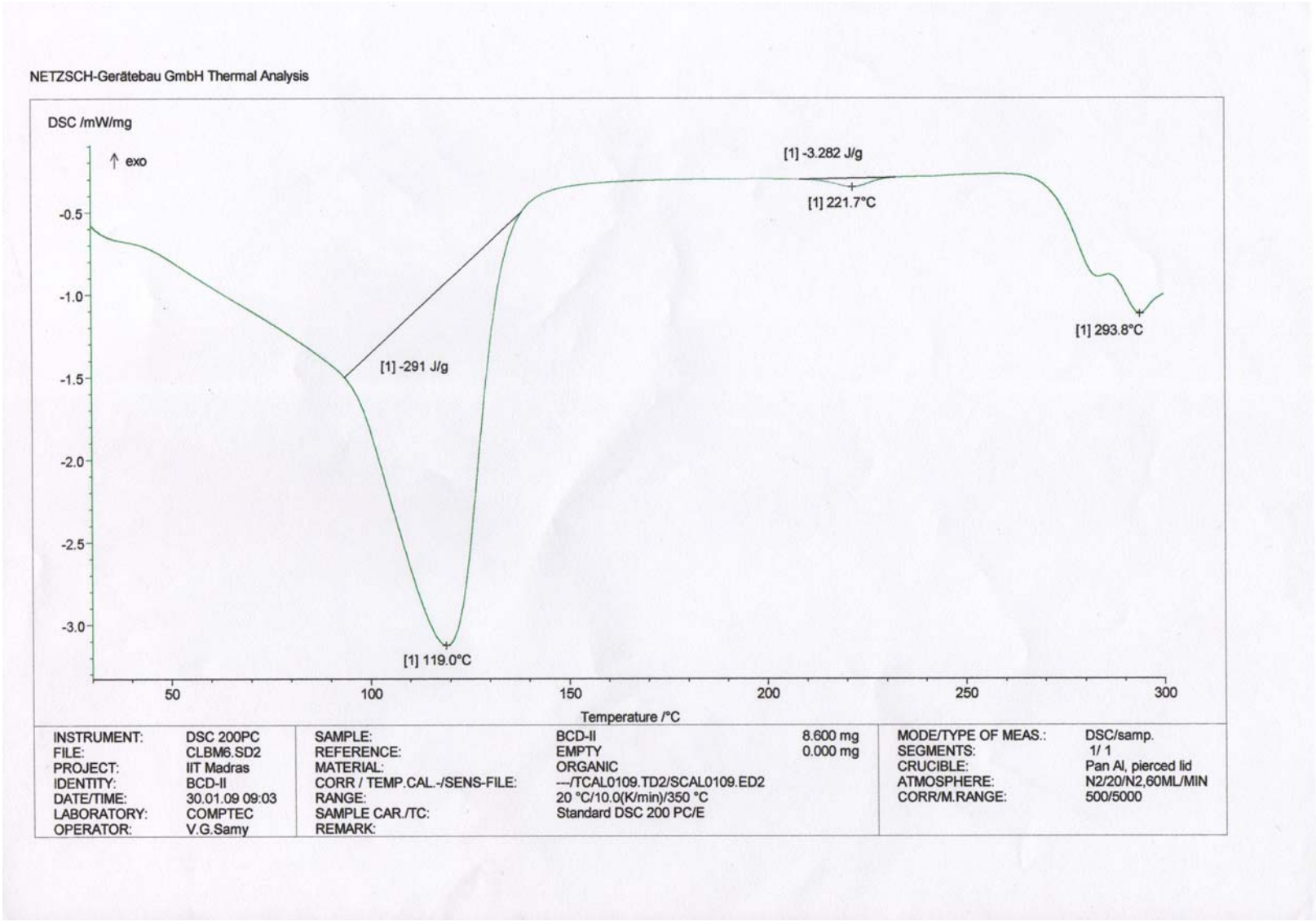


Fig 7.1c: DSC thermogram of Eudragit

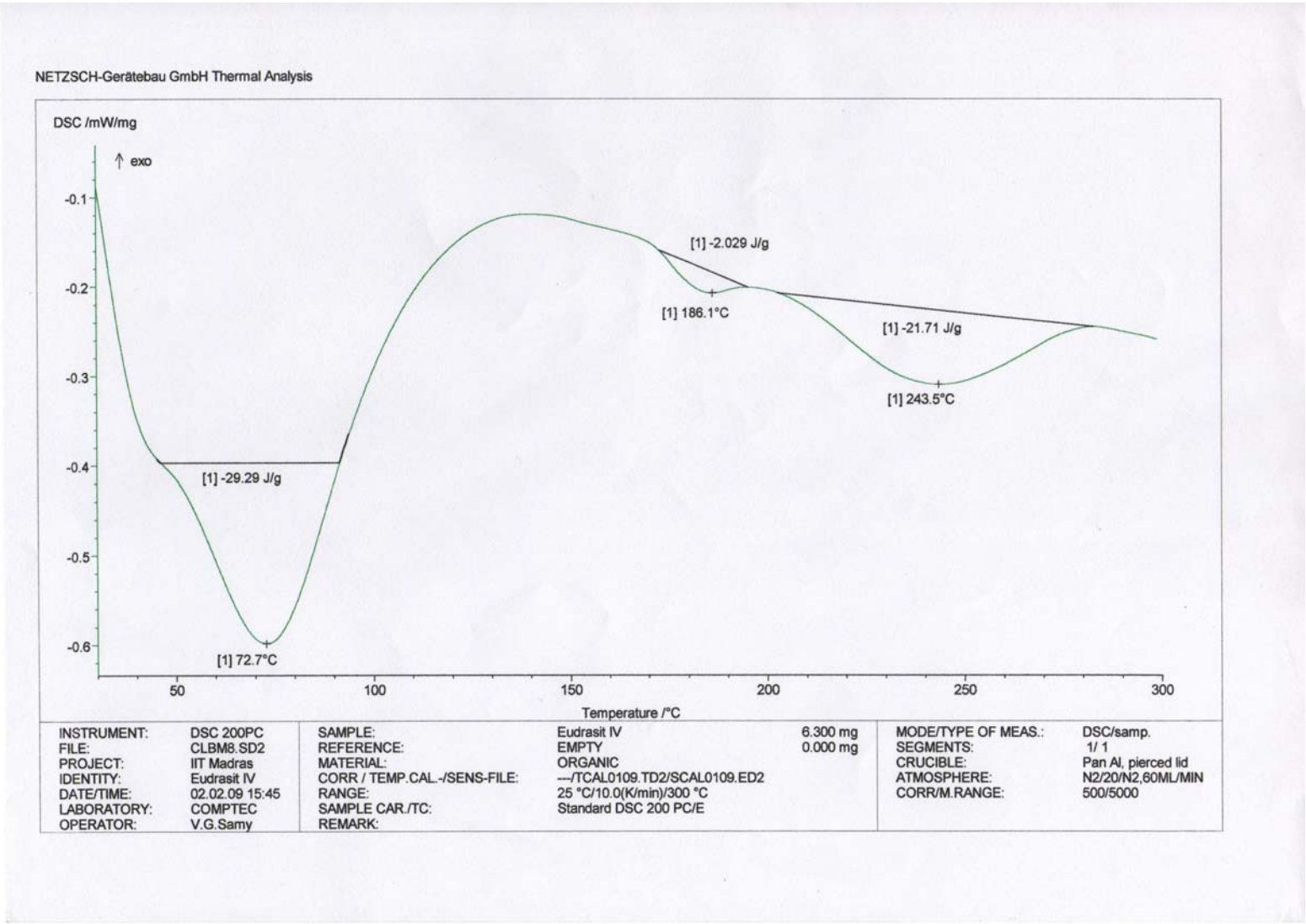
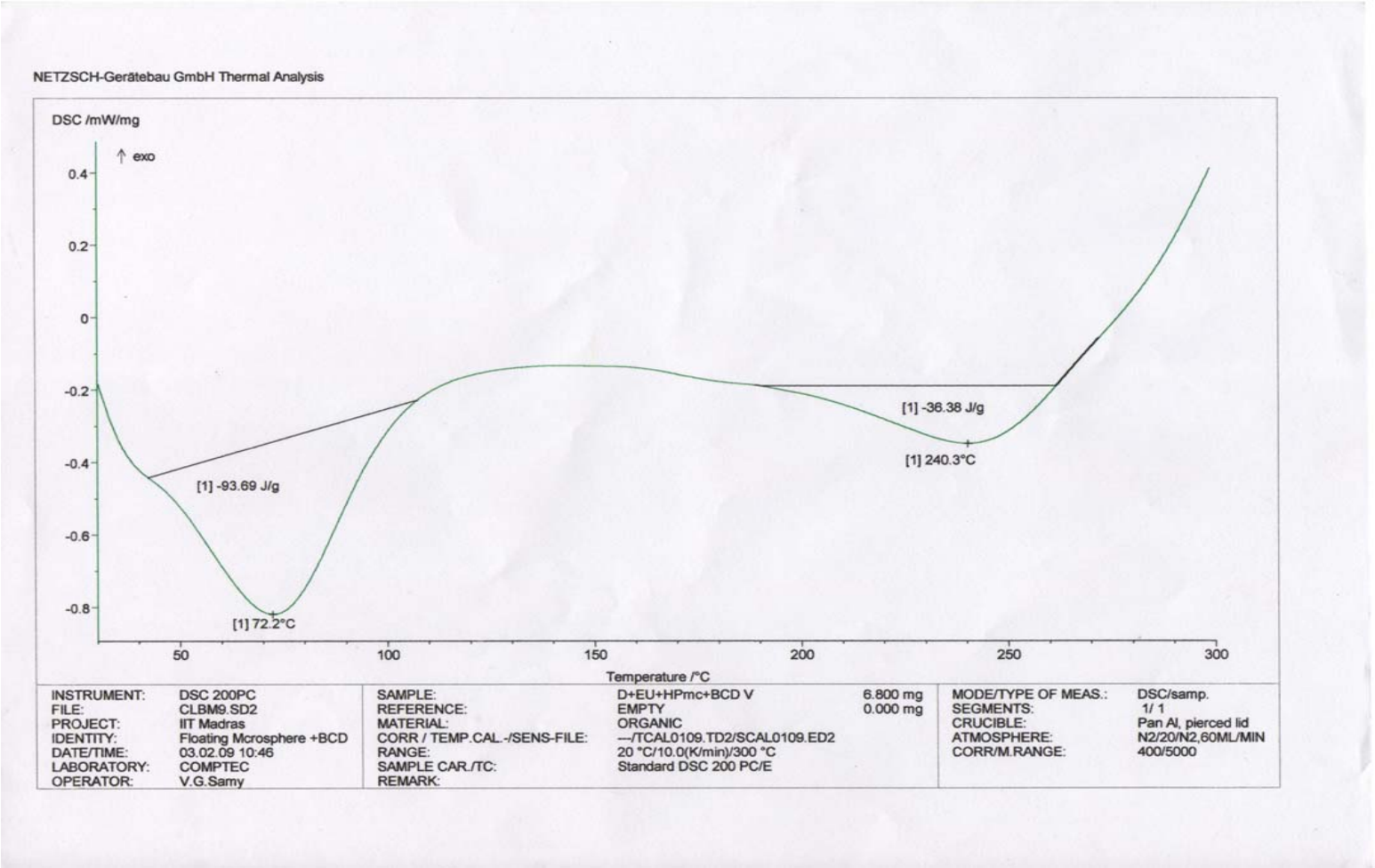


Fig 7.1d: DSC thermogram of formulation M 18



INVIVO EVALUATION OF THE SELECTED FORMULATIONS OF TABLET AND MICROSPHERES

◊ *INVIVO* FLOATING STUDY

X-ray analysis:

Approved by Institute of Animal Ethical Commitee

Protocol approval No.IAEC/XIII/11/CLBMCP/2008-2009 dated 11.12.08

The floating property of the selected tablet was studied by X-ray technique. Male rabbits with weight of 1.5 kg and with age of 12 months were selected. The animal was housed individually under environmental condition (25^0 C, 12 h light and dark cycle). The rabbit was fasted 36 h and allowed free access to water only.

***Table: 1 Batch* Formulae**

S.NO	ACTIVE INGREDIENTS	QUANTITY (mgs)
1	Barium Sulphate	24
2	Hydroxypropyl methyl cellulose KV600	12
3	Poly Ethylene Glycol 6000	12
4	Sodium Bicarbonate	12
5	PVP K-30	8
6	Lactose	8
7	Magnesium Stearate	3
8	Talc	3

The granules were prepared by wet granulation method[157] as per formulae given in Table 1.

The Barium sulphate, hydroxy propyl methyl cellulose K 100, poly ethylene glycol 6000, Sodium bicarbonate, were passed through mesh 40# separately and blended thoroughly.

- The wet mass was passed through sieve 16# and dried at 65°C for one hour to get the moisture content less than one.
- The blend was granulated with PVP K-30 solution, which was prepared by dissolving PVP K-30 in IPA.
- Magnesium Stearate and talc were passed through sieve 40# and blended with dried granules.
- The lubricated granules were compressed on Cadmach eight punch tablet machine.

The prepared tablet was administered to Rabbit by intubation method and subjected for Roentographic studies for 30 minutes, 1st h, 3rd h, 5th h and 8th h

The rabbit was administered with gastroretentive systems prepared as per procedure under table no.1. The tablet was administered orally by placing them in hollow polyethylene tube. The tube was inserted in to the mouth of rabbit and blown. X-rays were taken at interval of 30 min, 1 h, 3 h, 5h and 9h. The x ray photographs of gastro retentive system were shown in Fig1a – 1e.

◊ *IN VIVO* ANTITUMOUR ACTIVITY OF FORMULATIONS M 18 AND FXI A ON EHRLICH ASCITES CARCINOMA IN MICE

Animals

Swiss male albino mice (20-25 g) were procured from Sri Venkateswara Enterprises, Bangalore, India, and used throughout the study. They were housed in standard microlon boxes and maintained on a standard laboratory diet and water *ad libitum*.

Cells

EAC cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur, India. They were maintained by weekly intraperitoneal inoculation of 10^6 cells/mouse.[200]

Maintenance of EAC cell lines

The EAC cells were propagated in the peritoneal cavity of the mice by injecting 10^6 cells. The cells were aspirated aseptically from the developed tumor mice, during the log phase on the 15^{th} day of tumor transplantation using 18 gauge needles by withdrawing the fluid from the peritoneal cavity. The ascitic fluid was washed three times in phosphate buffer saline (PBS) and the cell pellet was resuspended in PBS. The cell suspension was diluted to get 10^6 cells / ml.

- **Effect of formulations (M 18 and FXI A) on survival time.**

Animals were inoculated at 1×10^6 cells/mouse on day 0, and treatment with **M 18 and**

FXI A started 24 h after inoculation, at doses of 20 mg/kg/d p.o. The control group was treated with the same volume of 0.9% sodium chloride solution. All the treatments were given for 9 days.

Ehrlich Ascites Carcinoma tumour (EAC) model

Four groups of swiss albino mice were taken. Each group contained six animals of both sex with body weight ranging from 25 – 35 g. the weight of the individual mouse in each group was recorded. They were injected with 1X 10^6 EAC cells intraperitoneally and housed in different polypropylene cages with paddy husk bedding and 12 hour, day and night cycle was maintained.

Group 1 served as tumor control and

Group II contained tumor bearing mice treated with 5 fluorouracil 20mg/kg body weight by oral route.

Group III and IV were treated with formulation F IXB and M 18 (20mg/kg)

Treatment was then started on the 11^{th} day of tumor induction and the administration was continued for five alternative days. The following parameters were studied and compared with control groups.

a) Body weight analysis

b) Mean survival time(MST)

c) Percentage increase in life span % ILS)

- Body weight analysis

All mice from the treatment group were weighed on 11^{th} day of tumor inoculation (i.e) on the day of commencement of drug administration. The average gain in body weight and % decrease in body weight was calculated by the following formula.

% Decrease in body weight = gain in body weight of control – gain in body weight of treated group/gain in body weight of control.

- Mean survival time

Mean survival time[201] (MST) of each group, containing 6 mice, was noted. The smear was prepared from ascites fluid on 14^{th} day and stained with Geimsa staining techniques. Average body weight was calculated. The antitumor efficacy of M18 and FIX A was compared with that of pure sample (5-Fluorouracil). MST of treated groups was compared with those of control groups by the following calculation:

$$\text{Increase in life span} = \frac{T - C}{C} \times 100$$

Where T = number of days, treated animals survived and

C = number of days, control animals survived.

❖ **Effect of formulations (M 18 and FXI A) on solid tumour**

Mice were divided into four groups (n=6). Tumour cells (1×10^6 cells / mice) were injected into the right hind limb of all the animals intramuscularly. Mice of group I were

tumour control. Group II, III & IV received 5 FU, M 18 and F IXA respectively (20 mg/kg) orally for 5 alternate days. Tumour mass was measured from 11^{th} day of tumour induction and was repeated every 5^{th} day for a period of 30 days. The volume of tumour mass was calculated using the formula $V = 4/3\ \pi\ r^2$ where r is the mean of r_1 and r_2 which are two independent radii of the tumour mass[202].

Statistical analysis

All the values were expressed as mean ± SEM. The data was statistically analyzed by one-way ANOVA followed by Dunnett test. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

◊ *INVIVO* FLOATING STUDY

X-ray analysis:

Floating property was determined by x-rays studies and the results are presented in fig1a-1e. The results showed that the tablet floated for 8 hours.

◊ *IN VIVO* ANTITUMOUR ACTIVITY OF FORMULATIONS M 18 AND FXI A ON EHRLICH ASCITES CARCINOMA IN MICE

Effect of formulations on mean survival time (MST) and Average increase in body weight

The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal.[203] The above results demonstrated the antitumour effect of M 18 and F IXA against EAC is Swiss albino mice. A significant ($P<0.01$) enhancement of MST was observed.

The effect of formulations on the survival of tumour bearing mice showed MST for the control group to be 20 days, while it was 34 days (78.94 %) and 37 days (94.73%) respectively for the group treated with formulation F IXA and M18 (20 mg/kg/d p.o). These results showed that MST was increased for both formulations, when compared to that for 5-FU, the Pure drug, for which the MST was 32 (68.42 %) (Table 2).

There is a tendency for increase in body weight in tumour bearing mice, which is the result of increased formation and collection of ascitic fluid. Potential anticancer drugs

decrease this increased body weight by decreasing the formation of ascites and this effect is due to the cytotoxicity effect against malignant cells, which induce ascites. Tumour bearing mice showed an average increase in body weight to the extent of 18.50 g. The increase in body weight was considerably less on treatment with formulations F IXA and M18 (20 mg/kg). The increased body weight was reduced significantly ($P<0.01$) to 6.68, 4.54 & 4.06 g respectively on treatment with pure sample, F IXA and M18 (Table 2).

Effect of Formulations on Solid tumor volume

Estimation of solid tumour volume is a direct method of evaluation of anticancer activity. It is indeed a suitable method, which does not involve sacrificing the animal. In the study, the tumour mass was directly measured after implantation intramuscularly. The solid tumour volume was increased by 6.15 ± 0.45. EAC bearing mice, treatment with M18 and F IXA decreased significantly ($P<0.01$), the tumour volume to 4.17 ± 0.13 and 4.36 ± 0.23 ml respectively at the end of 30 days (Table 3). The decreased tumour volume (4.63 ± 0.11) of pure drug treated group was less when compared to formulated one. The antitumour effect of F IXA and M18 is very well evident from these observations.

Cytological studies of ascitic fluid on the 14^{th} day in EAC bearing mice revealed that the tumour cells are large in size showing binucleation. In 5 FU (20 mg/kg) treated animals bearing EAC, the cells showed high n/c ratio, plasmocytoid feature with varying degree of degeneration and cytoplasmic vacuolation which is characteristic of immunoblast. The animals treated with Formulated M 18 and FIXA **(**20 mg / kg) bearing EAC cells, showed plasmacytoid feature with varying degree of degeneration and

cytoplasmic vacuolation and also showed active mitosis. All these cytological studies indicate the cytotoxic effect of the formulations (Fig 2a – 2d)

In EAC bearing mice, there was a regular and rapid increase in ascitic fluid volume. Ascitic fluid is the direct nutritional source for tumour growth; it meets the nutritional requirement of tumour cells.[204] The formulations M18 and F IXA treatment decreased the volume of solid tumour as well as ascites volume, decreased average bodyweight increase and increased the life span when compared with the plain drug.

It may conclude that **the gastroretentive formulations** decreased the nutritional fluid volume and thereby arrest the tumour growth and increase the life span. **The anti cancer efficiency of 5-FU loaded gastroretentive formulations are comparatively higher than the pure drug. This may help to reduce the total dose required for cancer therapy and ultimately, may in turn reduce the dose related systemic side effects which is the major goal and scope of this work.**

Table 2.Effect of M 18 and FIXA treatment on the survival and Average Body weight Changes of Tumour Bearing Mice

Treatment	Dose (mg/kg)	MST (d)	Life Span (%)	Average increase in body wt (g)
Tumour control	2 ml/kg	19 ± 1.10	-	18.50 ± 0.14
5FU	20	32 ± 1.25[a]	68.42	6.68 ±0.06 [a]
M 18	20	37 ± 1.36 [a]	94.73	4.06 ± 0.04 [a]
F IXA	20	34 ± 1.47 [a]	78.94	4.54 ± 0.08 [a]

n = 6 animals in each group; Days of drug treatment = 9

[a]P< 0.01 Vs Tumour control

Data were analyzed by using one way ANOVA followed by Dunnett test.

Table 3: Effect of M 18 and F IXA formulations on solid tumor volume

Treatment	Dose (mg/kg)	Solid tumor volume (ml)			
		15th day	**20th day**	**25th day**	**30th day**
Tumor control	-	3.78 ± 0.17	4.08 ± 0.18	5.74 ± 0.16	6.15 ± 0.45
5FU	20	2.98 ± 0.13[a]	3.45 ± 0.17	3.92 ± 0.14[a]	4.63 ± 0.11[a]
M 18	20	2.46 ± 0.10[a]	3.12 ± 0.43[b]	3.54 ± 0.32[a]	4.17 ± 0.13[a]
F IXA	20	2.79 ± 0.16[a]	3.29 ± 0.17	3.77 ± 0.36[b]	4.36 ± 0.23[a]

N = 6 animals in each group; Values are expressed as mean ± SEM.

[a]P<0.01; [b]P<0.05 Vs Tumour Control.

Data were analyzed by using one way ANOVA followed by Dunnett test.

***Fig:1 .IN VIVO* – STUDY –Determination of floating property by X – Ray Technique**

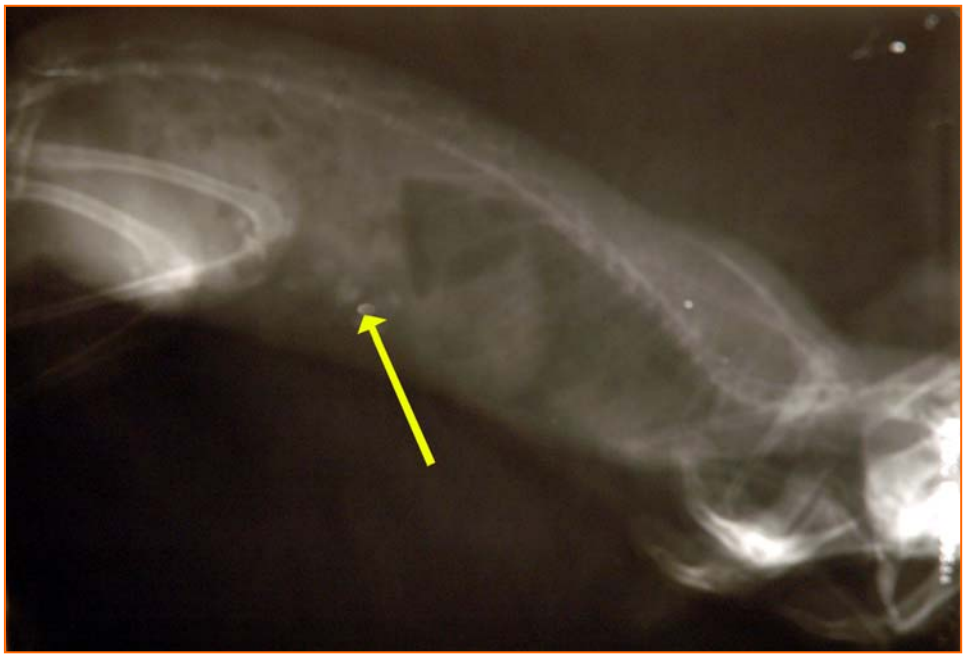


***Fig 1 a* – Floating property of formulation After 30 Minutes**

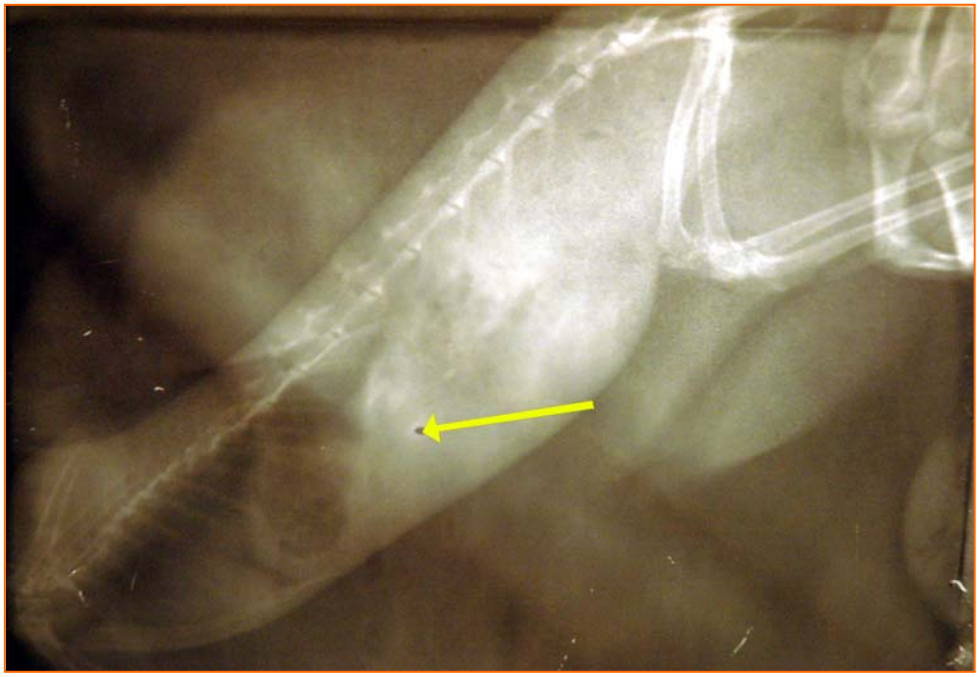


***Fig 1 b* - Floating property of formulation after 1 Hour**

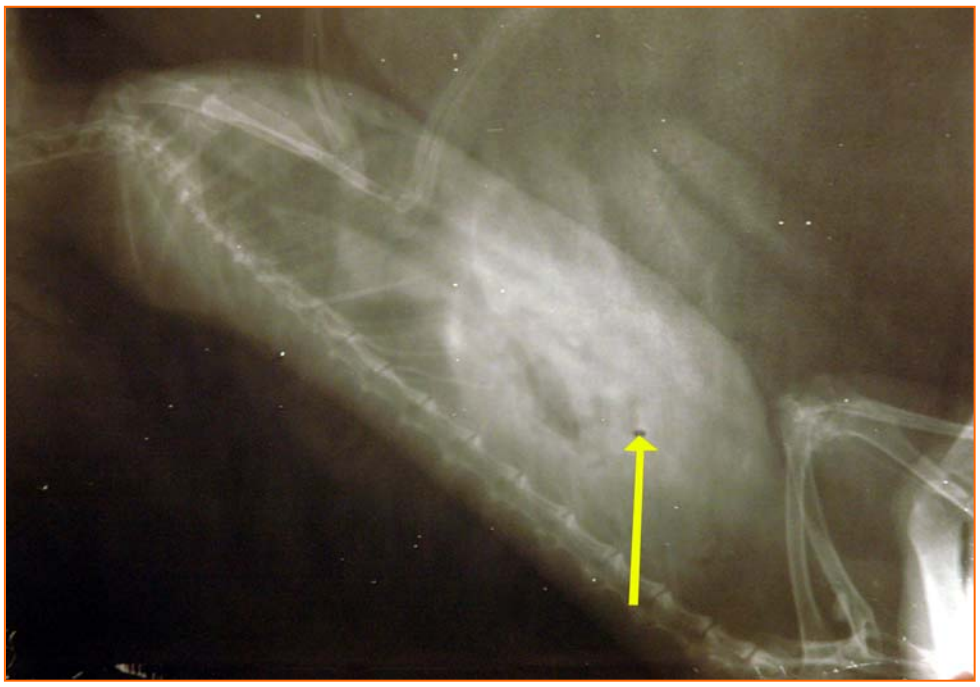


Fig 1 c **- Floating property of formulation After 3 Hours**

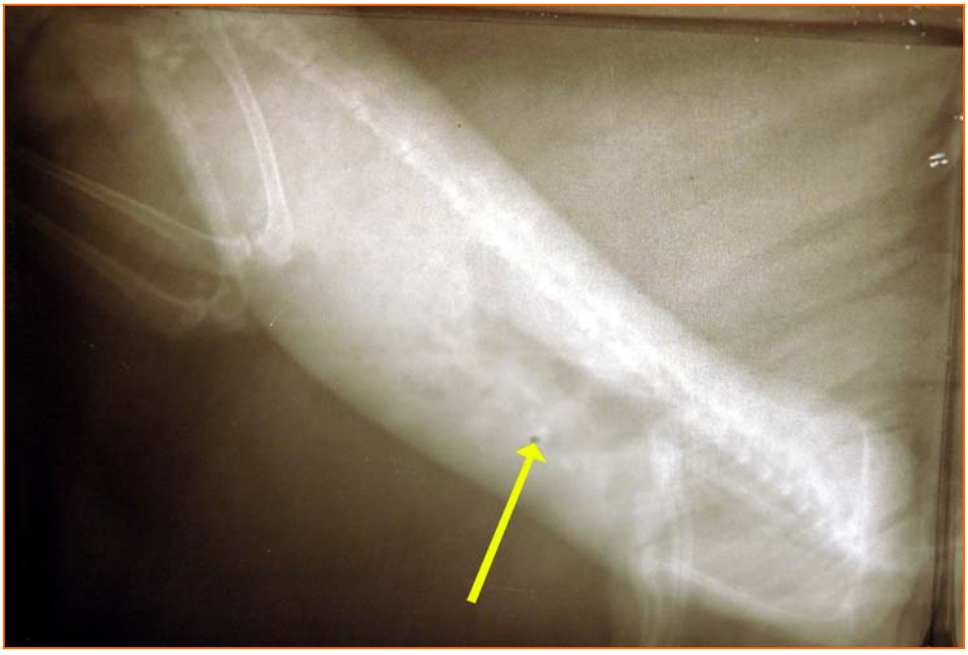


Fig 1 d **- Floating property of formulation After 5 Hours**

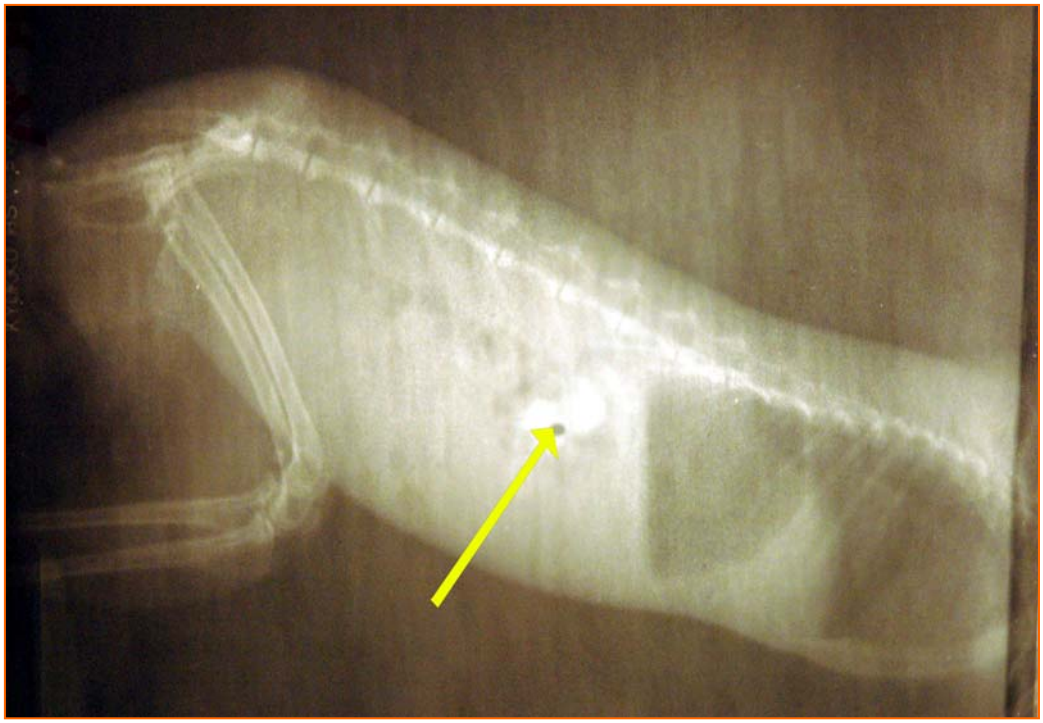


***Fig 1 e*- Floating property of formulation After 9 Hours**

Cytological Studies Shows Antitumour Activity of Formulations against Ehrlich Ascites Carcinoma

Fig 2a.

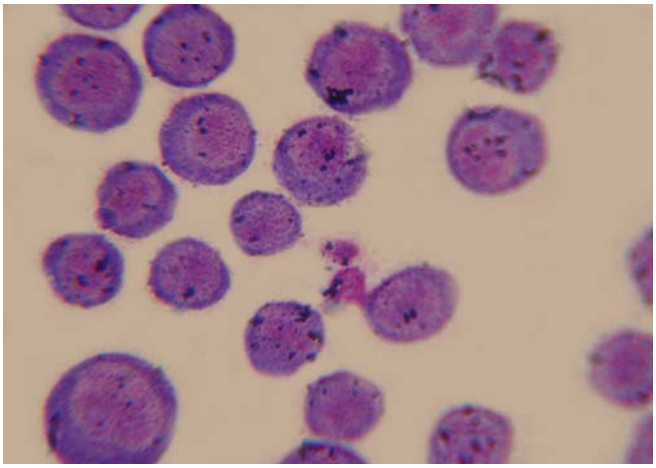


Fig 2a. Tumour cells are large in size. It shows sheet of lymphoma with nucleation. (**Tumour Control)**

Fig 2b

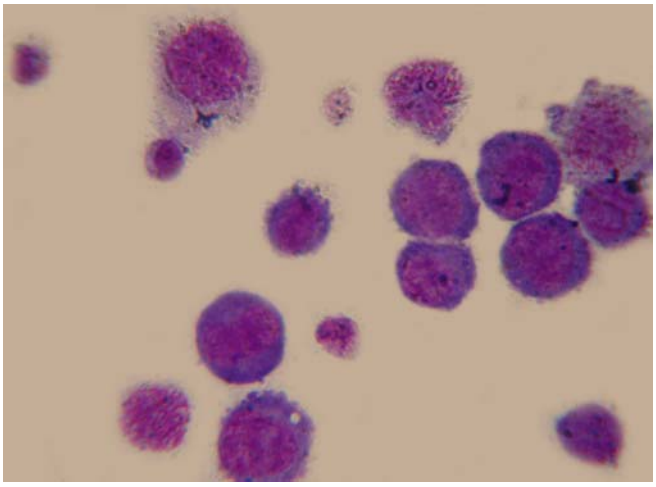


Fig 2b. Shows large lymphoma cell with high n/c ratio. Some of them show cytoplasmic vacuolation. (**Treated with 5 FU**)

Fig 2c

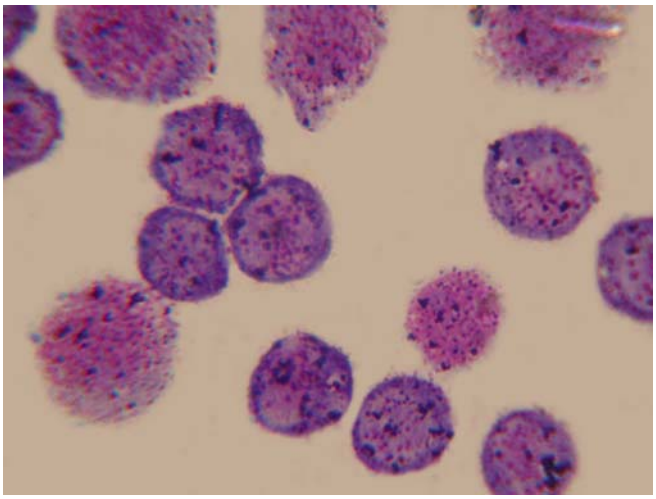


Fig 2c. Shows atypical mitosis with varying degree of degeneration and cytoplasmic vacuolation. (**Treated with IXA)**

Fig 2d

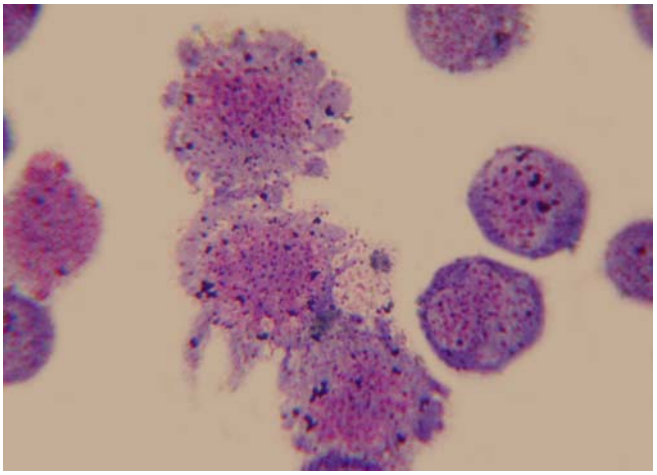


Fig 2d. Plasmacytoid feature with varying degree of degeneration and also showed active mitosis.(**Treated with F M18)**

STABILITY STUDIES

Stability studies of selected 5 FU GRS tablet formulation F- IX A and M18 was subjected to stability studies at 40º C under 75±5% relative humidity condition and kept for three-month stability studies. Samples were analyzed for color change in physical appearance, buoyancy, drug content, and dissolution parameters. The results are shown in the table 9.1 – 9.3 and represented in fig 9.1 and 9.2 respectively.

RESULTS AND DISCUSSION

From the result it was observed that there was no significant change in physiochemical properties as well as in drug release profile even after storage at 40ºC for three months. It may be inferred that there was no degradation and change in the matrix system**.** It was observed that there was no change in the morphology of microspheres as well as no agglomerates was formed. The percentage residual drug content of the formulations was found to be 98.29% for F IXB and 40.36% for M18 after storage for 3 months.

From the results it was observed that there was no significant change in physiochemical properties as well as in drug release profile even after storage at 40ºC for three months. It may be inferred that there was no degradation and change in the matrix system.

It was evident that formulation M18 with RS 100 and RLPO, HPMC K 100 and complexed drug and formulation F IXB with complexed drug and HPMC polymer was stable under storage conditions.

Table: 9.1. **Physical characteristics of 5 FU Floating (FIX-B) tablet after storage for three months at 40ºC**

S.NO	**Parameters**	**FIXB**	**M 18**
1	Physical appearance	No change	No change
2	Buoyancy Lag Time(seconds)	26	-
3	Percentage floating	-	84.16
4	Duration of Floating (hours)	24	24
5	% Drug content	98.29	40.36

Table 9.2: Percentage of drug retained after stability study

Sl.No.	Sample withdrawal day	% Potency retained(Mean ± S.E)	
		F IXB	M18
1	0 (Initial)	99.7 ± 1.04	41.36 ± 2.03
2	30	99.40 ± 4.084	41.0 ± 2.081
3	60	99.36 ± 3.384	40.85 ± 1.161
4	90	98.29 ± 0.452	40.36 ± 0.261

***Table: 9.3. In Vitro* Dissolution Study of 5 FU Floating tablets (F-IXB) after storage for two months @ 40° c**

Medium : Simulated Gastric Fluid pH 1.2

Apparatus : USP basket, Rpm : 100

S.No	Sampling Time (hrs)	Cumulative percentage released ± S.D M18	Cumulative percentage release ± S.E F IXB
1	1	23.98 ± 0.453	14.67 ± 1.78
2	2	34.79 ± 0.344	18.65 ± 1.98
3	3	49.96 ± 0.176	29.96 ± 2.65
4	4	59.98 ± 0.229	34.39 ± 1.27
5	5	65.34 ± 0.622	45.67 ± 3.61
6	6	69.91 ± 0.761	51.86 ± 2.11
7	7	78.39 ± 0.327	58.98 ± 3.31
8	8	80.60 ± 0.425	66.85 ± 2.66
9	9	86.73 ± 0.098	73.26 ± 1.89
10	10	92.74 ± 0.130	86.24± 2.26
11	12	95.99 ± 0.191	93.61 ± 1.28

Cumulative % Drug Release readings are mean ±SE of triplicate values

Fig 9.1: Comparison of *invitro* release profile of selected formulation (M18) after stability study

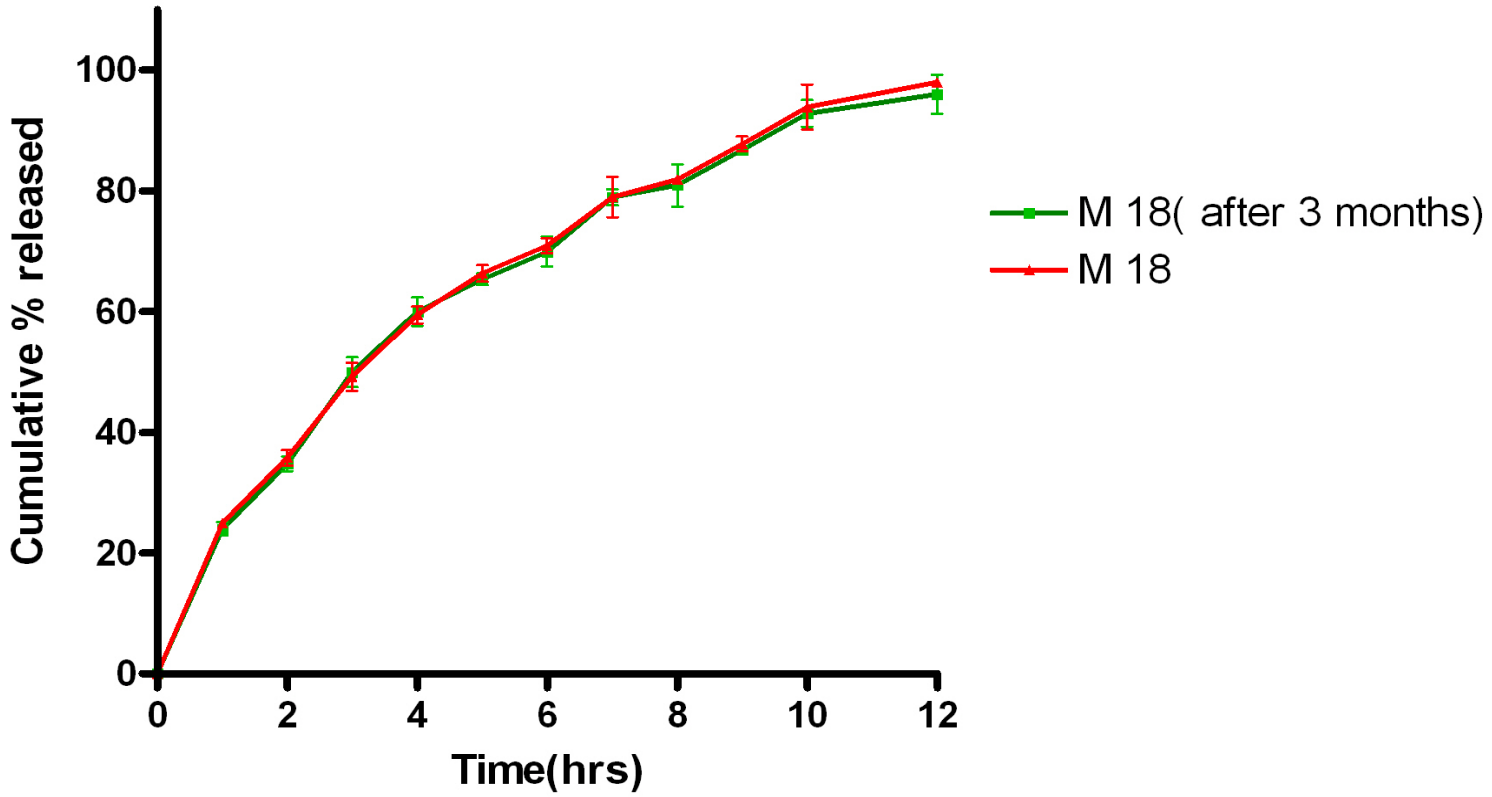
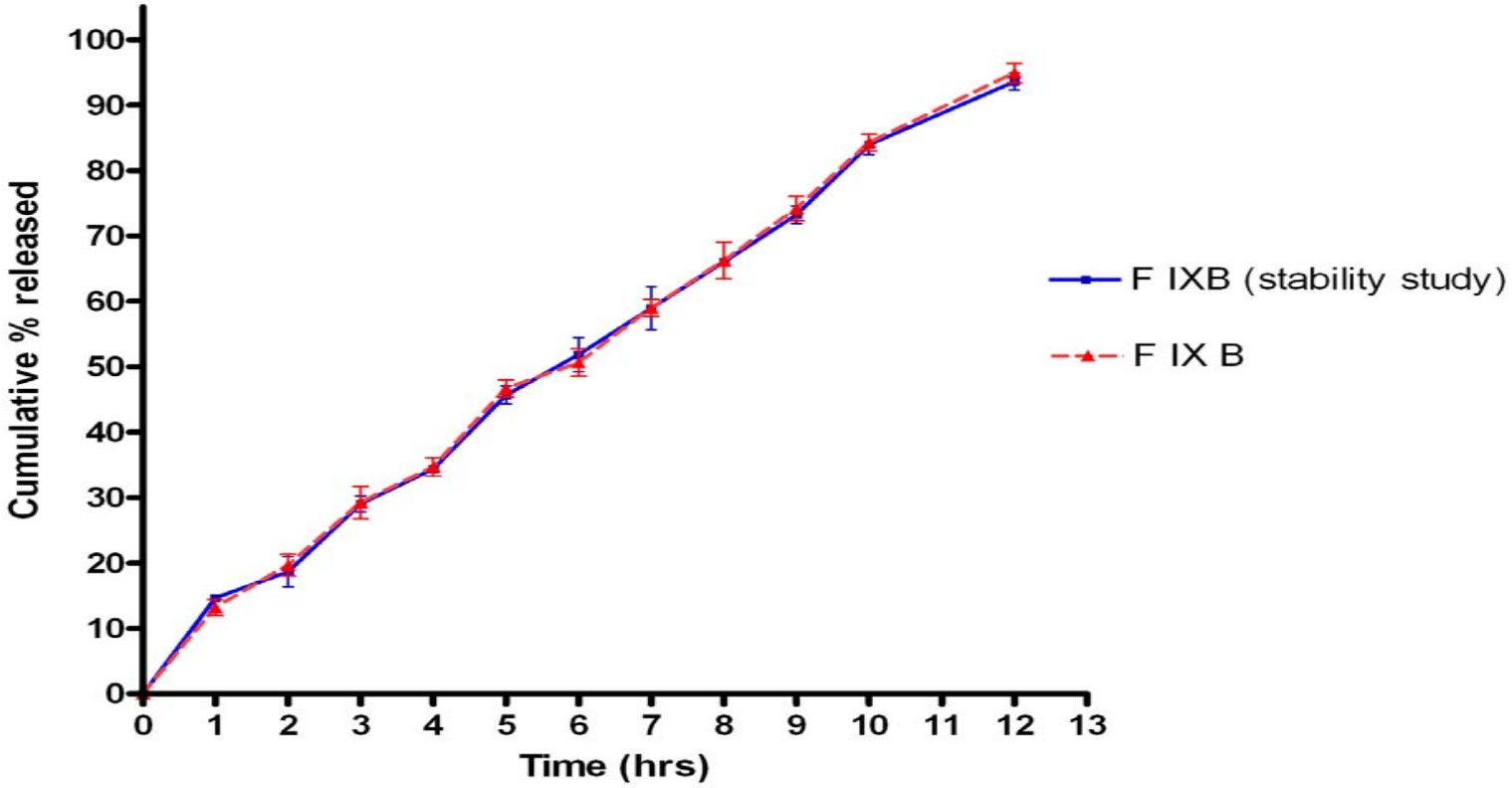


Fig 9.2: Comparison of *invitro* release profile of selected formulation after stability study



SUMMARY AND CONCLUSION

Targeted drug delivery systems are designed to release the medicament continuously in a predetermined pattern for a fixed period of time either systemically or to a specified organ to ensure safety and to improve its efficacy as well as patient compliance. Drug released from these drug delivery systems should be at a desired, predictable and reproducible rate. Gastroretentive system (GRS) is a topic of current interest in the design of controlled release drug delivery systems to target the drug to the site of absorption. It prolongs the residence time of the dosage form at the site of application or absorption and facilitates an intimate contact of the dosage form with the underlying absorption surface and thus contributes to improved and/or better therapeutic performance of the drugs. In the present investigation design of floating tablets (GRS) of fluorouracil for oral controlled release is aimed.

Fluorouracil is used in the treatment of cancer. It is sparingly soluble in water and its absorption is dissolution rate limited. Fluorouracil has a short biological half life and erratic absorption. To avoid the dose related toxicity and to prolong its duration of action controlled release products are necessary.

The first chapter deals with introduction about the clear advantage of controlled release dosage forms and the advantages derived with gastro retentive system. In this chapter absorption window, modulation of GI transit time, basic gastrointestinal tract physiology, factors affecting gastric retention, classification of floating drug delivery system, evaluation methods are discussed. Gastric cancer, its causes and the treatment were also discussed.

Chapter II contains literature review on the gastroretentive systems, fluorouracil and β cyclodextrin. Drug profile, polymer profile and excipient profile are given in chapter III. Preformulation studies of the drug were documented in chapter IV. The characterization of the drug material, 5 fluoro uracil that included physicochemical properties such as melting point, loss on drying, bulk and tapped density were done.

A compatibility study for polymer and drug was carried out to assess any interaction, by IR spectroscopy. The spectra showed no interaction had occurred with the excipients.

As fluorouracil is sparingly soluble in water, it was complexed with β cyclodextrin to enhance the solubility of the drug. The aqueous solubility and dissolution rate of 5-fluorouracil can be increased by inclusion complexation with β-cyclodextrin. Molecular-modeling studies support the formation of stable molecular inclusion complexation of 5-fluorouracil with β-cyclodextrin monomer (1:1). Complexes were prepared by physical mixture, kneading, co evaporation and freeze drying methods. Two ratios 1:1 and 1:2 were formulated. These eight complexes were subjected to Phase-solubility study, molecular modeling to confirm the ratio and dissolution study. The complexes formed were confirmed by DSC studies. Phase solubility profile indicated that the solubility of 5-fluorouracil increased in the presence of β-cyclodextrin monomer. Results obtained by different characterization techniques clearly indicate that the freeze-drying method leads to formation of solid state complexes between 5-fluorouracil and β-cyclodextrin. The complexation of 5-fluorouracil with β-cyclodextrin lends an ample credence for better therapeutic efficacy. Complexation and the enhancement of solubility are discussed in chapter V.

In the present work, a floating gastro retentive drug delivery system was developed and fabricated containing the drug 5 fluoro uracil. The aim of the work was to get a modified release pharmaceutical dosage form that could be used in the treatment of cancer. Floating drug delivery system are well proved and documented to be therapeutically superior to conventional dosage system in number of studies.

In chapter VI the optimization of the tablet process parameters was performed to find out the optimum operational conditions and to optimize the formula. The tablets were obtained by wet granulation method for all the formulations F- I to F- XII and evaluated for the buoyancy lag time and floating time, hardness, weight variation and drug content. Based on the performance with respect to buoyancy lag time, floating time and the release characteristics, the formulation (F- IX) was selected as the best formula as it showed a buoyancy time of 24 seconds and a floatation time of 24 hours. This formulation (F- 9) showed a sustained release rate throughout its release period.

Two batches F- IXA and F- IXB of the prototype formulations F- IX were prepared and studied for the reproducibility. It was found that the physiochemical properties remained intact and the release characteristic was more or less the same pointing to good reproducibility.

Chapter VII describes about the floating 5 fluorouracil microspheres which are multi-particulate matrix type system containing uniformly dispersed or dissolved drug.

Floating Microspheres were prepared by emulsion solvent evaporation process by using four different polymethacrylates having different permeability characteristics. The main objective of the preparation was to select the formulation which would provide optimum release till 12 hours and improve the gastric residence of the drug by staying in the stomach for long time.

The drug entrapment efficiency of the formulations (M1 to M16) was affected only by drug polymer ratio. This drug polymer ratio also influenced the particle size of the microspheres. This was due to the increase or decrease in the viscosity of dispersed phase in different ratio of drug and polymer. The percentage floating of the microspheres were above 70% which was found to be satisfactory.

The dissolution profiles of various microspheres containing different ratio of drug and polymer indicated that the release of 5 fluorouracil was more and faster from Eudragit RL100 polymer than Eudragit RS100. The combination of two different types of polymers having different permeabilities (RL and RS-type) in the same drug: polymer ratio would have almost same release rate. Burst effect was more in the microspheres containing drug: polymer ratio of 1:1 than 1:2 ratio.

The different release profiles for microspheres were fitted into the different models, zero order, first order, Higuchi model and Peppas model. It was revealed that most of the formulations followed Higuchi and Peppas model with $P < 0.001$.From the results the formulation M 8 was found to have desired properties with maximum release. Hence to check the reproducibility two batches M17 and M18 were prepared with the same formulation as that of M8 and HPMC. HPMC is a hydrophilic polymer. It was expected to have more controlled release of the drug. The formulation M17 and M18 were evaluated for floating behaviour, drug content and dissolution study.

It was revealed that floating microspheres containing 5 fluorouracil with desired micromeritic properties and varied matrix permeability can be prepared from polymethacrylates with different permeability characteristics. The improvement or retardation of drug release rate, demonstrated the feasibility of this formulation strategy for controlled delivery of 5 fluorouracil. Indirectly, the inclusion of slightly water soluble drug, 5 fluorouracil into the polymethacrylates increases its solubility.

The selected best formulations F IXB and M18 were subjected to *invivo* studies, in chapter VIII, like determination of *invivo* floating behavior and invivo anti tumor activity. The formulations were given to the rabbits by intubation method and the invivo floating behavior was studied by roentographic study. It was found to be floating inside the rabbit stomach as can be seen from the X Ray.

The invivo antitumour activity was performed in albino mice to show that the formulations were better than the plain dug 5 fluorouracil. The parameters studied were effect of formulations on EAC tumor model and effect of formulations on solid tumor model. The gain in the body weight analysis, % inhibition of life span and mean survival time were calculated and analyzed statistically. Cytological studies showed that the formulation M18 and F IXB showed sustained and better antitumor activity when compared to plain drug.

In chapter IX Stability studies were performed for the selected formulations at 40°C and 75 % RH for three months according to ICH guidelines. The drug release characteristics of the formulations were evaluated before and after storage and compared. The formulations were found to be stable during the storage period.

CONCLUSION

The following are the conclusions drawn from the results of the investigation.

The aqueous solubility and dissolution rate of fluorouracil were markedly enhanced by complexation with β cyclodextrin.

- The phase solubility diagram of FU-βCD was of B Type and the increase in solubility was due to the formation of 1:1 M complex. The complexes formed are adequately stable confirmed by DSC studies and Molecular modeling studies.

- Solid inclusion complexes of FU-βCD exhibited higher rates of dissolution than the plain FU.

- A 2.5 fold increase in the dissolution rate of FU was observed with FU-βCD (1:1) inclusion complexes prepared by freeze drying technique.

The floating effervescent tablets were formulated by employing cyclodextrin complexes, HPMC K 100 as Polymer and sodium bicarbonate as gas generating agent.

- Release of fluorouracil from these tablets depended on their composition of drug polymer ratio and the viscosity of the polymer.

- Drug release from these tablets was diffusion controlled (Higuchi) with fickian pattern and followed zero order kinetics. The SEM photomicrographs confirmed the diffusion pattern with erosion.

The floating microspheres were formulated with different types of polymethacrylates.

- Release of drug from the microspheres depended on the permeability of the different polymethacrylates.

- Drug release from the floating microspheres with better release (M18) showed a higuchi diffusion pattern and followed zero order kinetics.

The anti cancer efficiency of 5-FU loaded gastroretentive formulations are comparatively higher than the pure drug. This may help to reduce the total dose required for cancer therapy and ultimately, may in turn reduce the dose related systemic side effects which is the major goal and scope of this work**.**

It may be concluded that the formulation of floating effervescent tablets and floating microspheres of 5 fluoro uracil is feasible and may be manufactured with reproducible characteristics even from batch to batch. Therefore, a stomach – specific Gastro retentive tablet and microsphere of 5 fluorouracil were formulated and characterized. It was found to reduce the unwanted dose dependent side effects, at other sites, by targeted drug delivery to gastric tumors. This may serve as an alternative to painful intravenous therapy.

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