FORMULATION AND EVALUATION OF SITAGLIPTIN PHOSPHATE LOADED TRANSDERMAL MICROSPHERES GEL

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CERTIFICATE

CERTIFICATE

This is to certify that the dissertation entitled, **"Formulation And Evaluation Of Sitagliptin Phosphate Loaded Transdermal Microspheres Gel"** bonafide work done by **Mr. R. VIGNESH (Reg.No:261611308), Department of Pharmaceutics, College of Pharmacy, Madurai Medical College-20** in partial fulfillment of The Tamil Nadu Dr.M.G.R medical university rules and regulations for award of **MASTER OF PHARMACY IN PHARMACEUTICS** under my guidance and supervision during the academic year 2017–2018.

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DEDICATED
TO MY
FAMILY
MEMBERS
AND
WELL WISHERS

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CHAPTER – I

INTRODUCTION

INTRODUCTION

SUSTAINED RELEASE DRUG DELIVERY SYSTEM

Sustained release dosage form is designed to maintain constant levels of a drug in the patient's bloodstream by releasing the drug over an extended period. Maintaining constant blood levels of the drug in the bloodstream increases the therapeutic effectiveness of the drug. Drugs are defined precisely under some act**.(Mithal B.M.,2004)** It is the single active chemical entity present in a medicine that is used for diagnosis, prevention, treatment, cure of a disease.(**Tripathi K.D.,2001**) Yet to a common man they are just substances which are administered to win back an individual from the states of disease or ill health to the normal health. The drugs also had mysterious origins and hence the word drug was a form of the word called 'drogues' meaning of mysterious origin. The word disease can be expressed as a combination of 'dis' and 'ease' indicating absence of easiness or well-being or a feeling contrary to the feeling of health.

With many drugs the basic goal of therapy is to achieve a steady state blood or tissue level that is therapeutically effective and nontoxic for an extended period of time. The basic objective in dosage form design is to optimize the delivery of medication so as to achieve a measure of control of therapeutic effect in the face of uncertain fluctuations in the in-vivo environment in which drug release takes place. The appropriate dosage form and correct dose will ensure the maximum availability of the drug. The pharmaceutical industry provides a variety of dosage forms and dosage levels of particular drugs thus enabling the physician to control the onset and duration of drug therapy by altering the dose or mode of administration.**(Lachmann.L and Lieberman.H.A.,1987)**

Scope of Sustained Drug Delivery

Sustained release delivery systems are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose. A sustained release dosage forms leads to better management of the acute or chronic disease condition. The basic

rationale of a sustained drug delivery system is to optimize the bio-pharmaceutic, pharmacokinetic and pharmacodynamic properties of a drug in such a way that its utility is maximized through reduction in side effects and cure or control of condition in the shortest possible time by using smallest quantity to drug, administered by the most suitable route. This is usually accomplished by maximizing drug availability, i.e., by attempting to attain maximum rate and extent of drug absorption; however, control of drug action through formulation also implies controlling bioavailability to reduce drug absorption rates. Sustained release tablets and capsules are commonly taken only once or twice daily, compared with counterpart conventional forms that may have to take three or four times daily to achieve the same therapeutic effect.

The novel system of drug delivery offer a means of improving the therapeutic effectiveness of incorporated drugs by providing sustained, controlled delivery and/or targeting the drug to desired site**.(Brahmankar,D.M. and sunil B.Jaiswal., 2002)** Oral route has been the most popular and successfully used for sustained delivery of drugs because of convenience and ease of administration, greater flexibility in dosage form design and ease of production and low cost of such a system. The sustained release systems for oral use are mostly solid and based on dissolution, diffusion or a combination of both mechanisms in the control of release of drugs.

Sustained release can be achieved by,

• Incorporating the drug in a carrier system

• Altering the structure of the drug at the molecular level

• Controlling the input of the drug into the bio-environment to ensure a programmed and desirable bio-distribution. The primary objectives 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.

Advantages of Sustained Release Dosage Form

i. Frequency of drug administration is reduced.

ii. ii. Patient compliance can be improved, and drug administration can be made more convenient.

iii. The blood level oscillation characteristic of multiple dosing of conventional dosage forms is reduced, because a more even blood level is maintained.

iv. Implicit in the design of sustained release forms, is that the amount of drug administered can be reduced, thus maximizing availability with a minimum dose.

v. The safety margin of high-potency drugs can be increased, and the incidence of both local and systemic adverse side effects can be reduced in sensitive patients. **.(Brahmankar,D.M. and sunil B.Jaiswal., 2002)**

Disadvantages

i. Administration of sustained release medication does not permit the prompt termination of therapy.

ii. The physician has less flexibility in adjusting dosage regimens. This is fixed by the dosage form regimen.

iii. Sustained release forms are designed for the normal basis of average drug biologic half-lives. Consequently, disease states that alter drug disposition, significant patient variation, so forth are not accommodated.

iv. Economically more costly processes and equipment are involved in manufacturing many sustained release forms.

Factors to Be Considered for the Formulation of Sustained Release:

Physicochemical properties

a) Dose size: For a ideal sustained release formulation the dose size should be not more than 500mg.

b) Aqueous solubility: The drug should not be more water soluble or poorly soluble; moderate solubility is needed.

c) Partition co-efficient: Both lipophillic or hydrophilic drugs are difficult to process and should possess optimum partition co-efficient**.**

d) Molecule size: Large molecules show small diffusion co-efficient and may be difficult to place into a suitable sustained release system. Drugs of molecular weight 500-700 finds no difficult in processing.

e) Drug stability: It should be significantly stable over an extended period of time in the GIT. Generally non-potent drugs are more stable when formulated as sustained release form. It should not show high degree of plasma protein binding.

Route of administration

Some routes of administration exert a negative influence on drug efficacy especially during chronic administration. Many physiological constrains improved by the particular route i.e., GI mobility, blood supply, first pass metabolism.

Biological properties

a) Absorption: Drugs that are slowly absorbed or adsorbed with variable absorption rate are poor candidates for sustained release. It is assumed that the GI transit time of 10-12 hrs, to be ideal for sustained release.

b) Metabolism: Rapid metabolism leads to poor formulation in extended release.

c) Distribution: Drugs with high apparent volumes of distribution which in turn influences the rate of elimination for the drug are poor candidates for sustained release.

d) Duration of action Drugs with short half-life and high doses impose a constraint because of the dose size needed and those with a long half-life are inherently sustained.

e) Therapeutic index Drugs with narrow therapeutic range require precise control over the blood level of drug placing a constraint on sustained release.

f) Length of drug therapy Expected length of drug therapy to achieve control or curve of ailment is an important factor, in design of control release products.

g) Disease state: Pathophysiological state of subject plays an important part in the design of suitable controlled release delivery system**.(Leon lachmann., et al.,2002)** and **(Vyas S.P. and Roop.K., 2002)**

Formulation Methods of Achieving Sustained Drug Release:

All sustained release formulations employ a chemical or physical barrier to provide slow release of the maintenance dose. Many formulation techniques have been used to ‘build’ the barrier into the per-oral dosage form. These techniques include the use of coatings, embedding drugs in a wax, fat or plastic matrix, microencapsulation, chemical binding to ion-exchange resins and incorporation in an osmotic pump. The initial rapidly releasing priming dose of drug may be provided by incorporating that portion of the drug in a separate rapidly releasing form in the dosage form, for instance, as uncoated, rapidly releasing granules or pellets along with coated, slowly releasing granules or pellets in a tablet or hard gelatin capsule dosage form.

Alternatively, immediate and rapid release of the priming dose has been achieved by virtue of the position of that portion of the drug being at the surface of a porous wax or plastic matrix. The maintenance dose is provided by drug embedded deeper in the porous matrix.

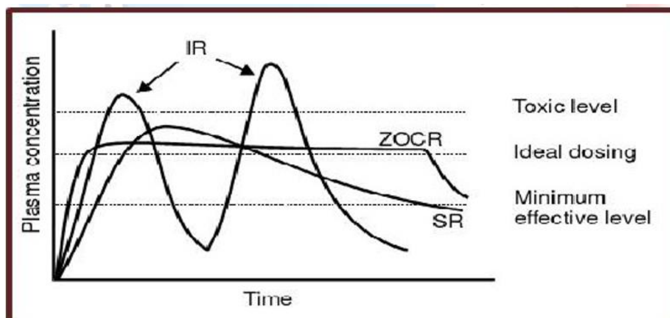


Figure 1.Characteristic representation of plasma concentrations of a conventional immediate release dosage form (IR), a sustained release dosage form (SR) and an idealized zero-order controlled release (ZOCR) dosage form (in combination with a start-up dose).

A repeat action tablet may be distinguished from its sustained release counterpart by the fact that the repeat action product does not release the drug contained therein in a slow controlled manner and consequently doesn't give a plasma concentration-time curve which resembles that of sustained release product9. A repeat action tablet usually contains two doses of drug, the first dose being released immediately following per-oral administration in order to provide a rapid onset of the therapeutic response. The release of the second dose is delayed, usually by means of an enteric coat. Consequently when the enteric coat surrounding the second dose is breached by the intestinal fluids, the second dose is released immediately. **(Lee V.H. and Robinson J.P.,1987)**

Classification

From a mathematical-modeling point of view, sustained-release systems may be classified according to the controlling physical mechanism(s) of release of the incorporated drug. We have proposed a convenient method based on the mechanism of transport for categorizing them as diffusion-controlled, swelling controlled, osmotically controlled, and chemically controlled systems. Sustained release of drugs, proteins, and other bioactive agents can be achieved by incorporating them either in dissolved or dispersed form in polymers.

Diffusion-Controlled drug delivery system: Diffusion is the most common mechanism for controlling the release. There are two major types of diffusion-controlled systems; reservoir devices and membrane devices. Drug release from each type of system occurs by diffusion through the macromolecular mesh or through the water-filled pores.

a) Reservoir systems

Reservoir systems consist of a polymeric membrane surrounding a core containing the drug. The rate-limiting step for drug release is diffusions through the outer membrane of the device. To maintain a constant release rate or flux of drug from the reservoir, the concentration difference must remain constant. This can be achieved by designing a device with excess solid drug in the core. Under these conditions, the internal solution in the core remains saturated. This type of device is an extremely useful device as it allows for time - independent of zero-order release. The major drawback of this type of drug delivery system is the potential for catastrophic failure of the device.

b) Matrix system

In matrix devices, the drug is dispersed throughout the three-dimensional structure of the polymer. Release occurs due to diffusion of the drug throughout the macromolecular mesh or water-filled pores. In these systems, the release rate is proportional to time to the one-half power. This is significant in that it is impossible to obtain time independent of zero-order release in this type of system with simple geometries.

C) Swelling –Controlled release systems: In swelling-controlled release systems, the drug is dispersed within a glassy polymer. Upon contact with biological fluid, the polymer begins to swell. No drug diffusion occurs through the polymer phase. As the penetrant enters the glassy polymer, the glass transition temperature of the polymer is lowered allowing for relaxations of the macromolecular chains. The drug is able to diffuse out of the swollen, rubbery area of the polymers. This type of system is characterized by two moving fronts:

the front separating the swollen (rubbery) portion and the glassy regions which moves with velocity, υ, and the polymer-fluid interface. The rate of drug release is controlled by the velocity and position of the front dividing the glassy and rubbery portions of the polymer.

For true swelling-controlled release systems, the diffusion exponent, n, is 1. This type of transport is known as Case II transport and results in zero-order release kinetics. However, in some cases, drug release occurs due to a combination of macromolecular relaxations and Fickian diffusion. In this case, the diffusion exponent is between 0.5 and 1. This type of transport is known as anomalous or non-Fickian transport.

d) Chemically-Controlled release systems

There are two major types of chemically controlled release systems: erodible drug delivery systems, and pendent chain systems in erodible systems, drug release occurs due to degradation or dissolution of the polymer. In pendent chain systems, the drug is affixed to the polymer backbone through degradable linkages. As these linkages degrade, the drug is released.

e) Erodible drug delivery systems: Erodible drug delivery systems, also known as degradable or absorbable release systems, can be either matrix or reservoir delivery systems. In reservoir devices, an erodible membrane surrounds the drug core. If the membrane erodes significantly after the drug release is complete, the dominant mechanism for release would be diffusion. Predictable, zero-order release could be obtained with these systems. In some cases, the erosion of the membrane occurs simultaneously with the drug release. As the membrane thickness decreased due to erosion, the drug delivery rate would also change. Finally in some erodible reservoir devices, the drug diffusion in the outer membrane does not occur. Under these conditions, drug release does not occur until the outer membrane erodes completely. In this type of device, the entire contents are released in a single, rapid burst.

For erodible matrix devices, the drug is dispersed within the three-dimensional structure of the polymer. Drug release is controlled by drug diffusion through the gel of erosion of the polymer. In true erosion-controlled devices, the rate of drug diffusion is significantly slower than the rate of polymer erosion, and the drug is released as the polymer erodes.

Environmentally responsive systems

Environmentally responsive materials show drastic changes in their swelling ratio due to changes in their external pH, temperature, ionic strength, nature and composition of the swelling agent, enzymatic or chemical reaction, and Electrical or magnetic stimulus In most responsive networks, a critical point exists at which this transition occurs. Responsive materials are unique in that there are many different mechanisms for drug release and many different types of release systems based on these materials. For instance, in the most cases drug release occurs when the gel is highly swollen and is typically controlled by gel swelling, drug diffusion, or a coupling of swelling and diffusion. However, in a few instances, drug release occurs during gel syneresis by a squeezing mechanism. Also, drug release can occur due to erosion of the polymer caused by environmentally responsive swelling. Another interesting characteristic about many responsive gels is that the mechanism causing the network structural changes can be entirely reversible in nature.

The ability of these materials to exhibit rapid changes in their swelling behavior and pore structure in response to changes in environmental conditions lends these materials favorable characteristics as carriers for bioactive agents, including peptides and proteins. This type of behavior may allow these materials to serve as self-regulated, pulsatile drug delivery system.

Initially, the gel is in an environment in which no swelling occurs. As a result, very little drug release occurs. However, when the environment changes and the gel swells rapid drug release occurs (either by Fickian diffusion, anomalous transport, or Case II transport). When the gel collapses as the environment

changes, the release can be turned off again. This can be repeated over numerous cycles. Such systems could be of extreme importance in the treatment of chronic diseases such as diabetes. . **(Lee V.H. and Robinson J.P.,1987)**

DIABETES:

Diabetes Mellitus (DM), often simply referred to as diabetes, is a group of metabolic diseases in which a person is mainly characterized by hyperglycemia either because of insulin deficiency or because of the resistance shown by the cells to insulin produced in the body. It may also be characterized by glycosuria, negative nitrogen balance, and sometimes ketonemia. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Diabetes Mellitus is classified based on the cause or mode of treatments into the following types:

1. Insulin-dependent diabetes mellitus (IDDM)

2. Non-insulin-dependent diabetes mellitus (NIDDM)

3. Gestational diabetes mellitus (GDM)

4. Secondary to other conditions

A) Type I (or) Insulin-dependent diabetes mellitus:

Characterized by the body's failure to produce insulin due to the destruction of β cells in the islets of langerhans, and requires the person to inject insulin.formerly, it is known as "juvenile diabetes," because it represents a majority of the cases in children, teenagers, or young adults, but it can also affect adults. Type-1diabetes is mostly caused by autoimmune disorder AND develops because the body immune system mistakenly destroys the beta cells in the islet tissue of the pancreas that produce insulin due to environmental factors. For the treatment of type I insulin must be given subcutaneously or by injecting through any other novel routs of administration.

B) Type II (or)Non-insulin-dependent diabetes mellitus:

Characterized by insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. People can develop type 2 diabetes at any age even during childhood. This form of diabetes usually occurs because of abnormality in gluco receptor of β cells, Reduced sensitivity of peripheral tissues to insulin, Excess of hyperglycemic hormones.Insulin is not sufficient for the treatment of type II diabetes.

C) Secondary to other conditions

Diabetes occurring as secondary to the conditions like Pancreatic disease, hormonal disease, Drug or chemical exposure, Insulin receptor abnormalities, certain genetic syndromes.

Signs and Symptoms :

The classical symptoms of diabetes are,

Polyuria (frequent urination),

Polydipsia (increased thirst)

Polyphagia (increased hunger).

Diabetic ketoacidosis.

Management

Diabetes mellitus is a chronic disease which cannot be cured except in very specific situations. Management keeps blood sugar levels as close to normal as possible, without causing hypoglycemia. This can usually be accomplished withdiet, exercise, and use of appropriate medications (insulin in the case of type 1diabetes, oral antidiabetec medications as well as possibly insulin in type 2diabetes.

Novel Oral Antidiabetecs

Dipeptidyl Peptidase (DPP)-4 Inhibitors

DPP4 inhibitors such as Sitagliptin and Vildagliptin are novel agents for treatment of type 2 diabetes. They work by improving β-cell sensitivity to glucose, where by it increases glucose-dependent insulin secretion. Gliptins can be used as Mono therapy or combined with metformin or sulfonyl ureas.. Gliptins are largely weight neutral.

Examples: Sitagliptin, Vidagliptin.

MICROENCAPSULTION:

Microencapsulation is a process whereby small discrete solid particles or small liquid droplets are surrounded and enclosed by an intact shell. Microencapsulation is used to modify and delayed drug release form pharmaceutical dosage forms. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a particular drug.

It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.

Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor. The intent of the paper is to highlight the potential of microencapsulation technique as a vital technique in novel drug delivery.

The Reasons for Microencapsulation

The reasons for microencapsulation are in some countless cases, the core must be isolated from its surroundings, as in vitamins from the deterioration a volatile core, improving the handling of a sticky material, or isolation core from chemical a attack. The problem may be as simple as masking the taste or odor of

the core, or as complex as increasing the selectivity of an adsorption or extraction process.

Fundamental Considerations

The realization of the potential that microencapsulation offers involves a basic understanding of the general properties of microcapsules, such as the nature of the core and coating materials, the stability and release characteristics of the coated materials and the microencapsulation methods.

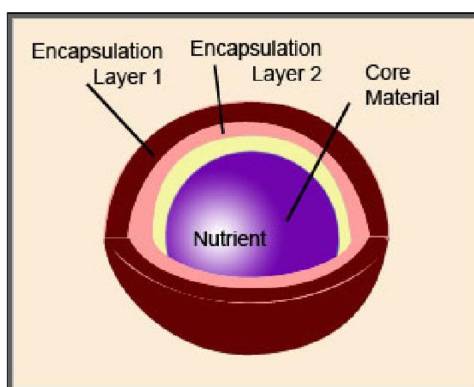
Core Material

The core material, defined as the specific material to be coated, can be liquid or solid in nature. The composition of the core material can be varied as the liquid core can include dispersed and/or dissolved material. The solid core can be mixture of active constituents, stabilizers, diluents, excipients and release-rate retardants or accelerators. The ability to vary the core materials composition provides definite flexibility and utilization of this characteristic often allows effectual design and development of the desired microcapsules properties.

Coating Material

The selection of appropriate coating material decides the physical and chemical properties of the resultant microcapsules/microspheres.

FIGURE-2 Encapsulation



While selecting a polymer the product requirements i.e. stabilization, reduced volatility, release characteristics, environmental conditions, etc. should be taken into consideration. The polymer should be capable of forming a film that is cohesive with the core material. It should be chemically compatible, non-reactive with the core material and provide the desired coating properties such as strength, flexibility, impermeability, optical properties and stability. Generally hydrophilic polymers, hydrophobic polymers (or) a combination both are used for the microencapsulation process. A number of coating materials gelatin have been used successfully; examples of these include polyvinyl alcohol, ethyl cellulose, cellulose acetate phthalate and styrene maleic anhydride. The film thickness can be varied considerably depending on the surface area of the material to be coated and other physical characteristics of the system. The microcapsules may consist of a single particle or clusters of particles. After isolation from the liquid manufacturing vehicle and drying, the material appears as a free flowing powder. The powder is suitable for formulation as compressed tablets, hard gelatin capsules, suspensions, and other dosage forms**. (C. Berkland., 2001)**

Multi-particulate Drug Delivery Systems:

Multi-particulate drug delivery system applies specially to multiple particles such as pellets, beads, microspheres, microcapsules. In recent years, multi-particulate dosage forms or microparticles have gained in popularity for a variety of reasons. Considerable research efforts have been spent on oral sustained or controlled release multi-particulate drug delivery system due to its advantages over monolithic dosage forms.

Multi-particulate drug delivery systems are mainly oral dosage forms consisting of a multiplicity of small discrete units, each exhibit some desired characteristics. In these systems, the dosage of the drug substances is divided on a plurality of subunit, typically consisting of thousands of spherical particles with diameter of 0.05-2.00mm.

Thus multi-particulate dosage forms are pharmaceutical formulations in which the active substance is present as a number of small independent subunits. To deliver the recommended total dose, these subunits are filled into a sachet and encapsulated or compressed into a tablet. **(C. Berkland., 2001)**

CHAPTER – II

MICROSPHERES-
A REVIEW

MICROSPHERES – A REVIEW

INTRODUCTION OF MICROSPHERES

Introduction:

An ideal controlled drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. The concept of miroencapsulation was initially utilized in carbonless copy papers. More recently it has received increasing attention in pharmaceutical and biomedical applications. The first research leading to the development of micro-encapsulation procedures for pharmaceuticals was published by Bungenburg de Jong and Kass in 1931 and dealt with the preparation of gelatin spheres and the use of gelatin coacervation process for coating. In the late 1930s, Green and co-workers of National cash register co. Dayton, Ohio, developed the gelatin coacervation process. Since then may other coating materials and processes of application have been developed by the pharmaceutical industry for the Microsphere of medicines. Over the last 25 years numerous patents have been taken out by pharmaceutical companies for microencapsulated drugs. Microsphere is a rapidly expanding technology. As a process, it is a means of applying relatively thin coating to small particles of solids or droplets of liquids and dispersions.**(Lachmann.L and Lieberman.H.A.,1987)**

Microspheres are defined as 'solid spherical particles containing dispersed drug in either solution or microcrystalline form.' They are ranging in size from 1 to 1000 micrometer. Microspheres are in strict sense, spherical solid particles. Microcapsules are small particles that contains an active agent as a core material and coating agent as shell, at present there is no universally accepted size range that particle must have in order to be classified as microcapsules. However, many workers classify capsules smaller than 1 micrometer as nanocapsules and capsules layer more than 1000 micrometer as macroparticles. Commercial microcapsules typically have a diameter between 3-80 micrometer and contain 10-90 weight % cores. Microsphere is a rapidly expanding technology. It is the

process of applying relatively thin coatings to small particles of solids or droplets of liquids and dispersions. Microsphere provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials**(Chien YW.,1990)and(Asha P..,2006)**. Microsphere is receiving considerable attention fundamentally, developmentally and commercially. However the terms microcapsule and microspheres are often used synonymously. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer. Solid biodegradable microcapsules incorporating a drug dispersed or dissolved throughout the particle matrix have the potential for the controlled release of drug.

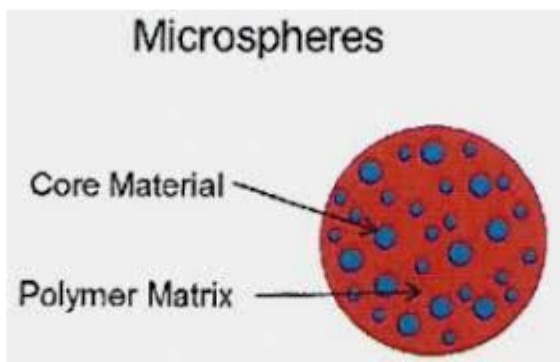


Figure-3 (Microencapsulation core material)

A wide range of core materials have been encapsulated including adhesives, agrochemicals, live cells, active enzymes, flavor fragrances, pharmaceuticals, and inks. Most capsule shell materials are organics, polymers, but fats and waxes are also used. Microcapsules can have a variety of structures some have a spherical geometry with a continuous core region surrounded by a continuous shell as other have an irregular geometry and contain a number of small droplets as particles of core material. Microcapsules are used in a wide range of oral and injected drug formulation. Encapsulated adhesive resins coated on automotive fasteners are routinely used to assure that such fasteners are firmly set when installed. Microcapsules are also the basis for a number of long acting commercial

pesticides and herbicides. Improvement of these products and development of new ones is an ongoing process that involves a large number of development groups globally.

Merits of Microsphere:

Microsphere offers the ability to do the following.

1. Microsphere change liquid to solid (powder as particles.) E.g. Clofibrate.
2. Microsphere separate reactive materials from one another and handle them in a mix. As store them for a long time. E.g. Mix of Aspirin and Chlorfilaramine.
3. Microsphere curtails any color, taste, odor or toxicity generates by core materials.
4. Microsphere protects materials from environment for examples by preventing oxidization. E.g. Vitamin A Palmitate.
5. Microsphere improves the effective life.
6. Microsphere holds a liquid on a flat surface by in effect, changing it to a solid.
7. Microsphere controls the releasing conditions. For example, we can control the timing of dissolving, volatilization, coloring, release of smell, mixing and reaction by changing the size of the capsules, the ratio of core materials to shell materials, or the properties of the shell materials, such as strength and permeability or by adding supplementary materials to core or shell materials.
8. Microsphere changes the specific gravity.
9. Microspheres make core materials easy to handle.
10. Microspheres solidify tacky materials and increase its fluidity. E.g. thiamine HCL, riboflavin
11. Microspheres are normally used to enhance material stability, reduce adverse or toxic effects as extend material release for different applications in various fields of manufacturing.

Demerits of Microsphere:

1. Drug entrapment is low because some portion of drug is lost in the dispersion vehicle.
2. The industrial scale of microspheres formulation is difficult because to maintain size of microspheres at industry level is difficult.
3. The manufacturing of microspheres involves use of solvents which make the process costly.
4. As compared to the extended release tablets and capsules, the manufacturing of microspheres is much more complicated.
5. Time consuming process as much time period for required for emulsification, vaporization of solvent and rigidization of microspheres.

Prerequisites for Ideal Microparticulate Carriers

The materials utilized for the preparation of microparticulates should ideally fulfill the following prerequisites:

- Longer duration of action
- Control of content release
- Increase of therapeutic efficiency
- Protection of drug
- Reduction of toxicity
- Biocompatibility
- Sterilizability
- Relative stability
- Water solubility or dispersability
- Bioresorbability
- Targetability
- Polyvalent

Fundamental Consideration:

Microsphere often involves a basic understanding of the general properties of microcapsules, such as the nature of the core and coating materials, the stability

and release characteristics of the coated materials and the Microsphere methods. The intended physical characters of the encapsulated product and the intended use of the final product must also be considered. **(Leon Lachman et.al)**

A.Core material

The core material, defined as the specific material to be coated, can be liquid or solid in nature. The composition of the core material can be varied as the liquid core can include dispersed and/or dissolved material. The solid core can be a mixture of active constituents, stabilizers, diluents, excipients and release rate retardants or accelerators. The core material plays a significant role in the production of microcapsules the core material defined as the specific material to be coated, can be liquid or solid in nature. It dictates the process as well as the polymer used as the coating material. It should be insoluble and non reactive with the coating material and the solvent for it water soluble and insoluble solid, water insoluble liquid solution and dispersions of liquids can be microencapsulated.

B. Coating material:

Coating material should be capable of forming a film that is cohesive with the core materials, be chemically compatible and non reactive with the core material and provide the desired coating properties such as strength, flexibility impermeability, optical properties and stability. The total thickness of the coatings achieved with Microsphere techniques is microscopic in size. The selection of the appropriate coating material dictates, to major degree, the resultant physical & chemical properties of the microcapsules, and consequently, this selection must be given due consideration. The coating material should be capable of forming a film that is cohesive with the core material; be chemically compatible and non-compatible and non reactive with the core material; and provide the desired coating properties, such as strength, flexibility, impermeability, optical properties and stability. The coatings materials used in Microsphere methods are available; to some extend, to in-situ modification. For example, colorants may be added to achieve product elegance or masking, or coatings may be plasticized or

chemically altered through cross-linking, for instance to achieve controlled dissolution or permeability. **(Jackson. And Lee, 1991).**

C. Selected stability, release and other properties

Three important areas of current Microsphere application are the stabilization of core materials, the control of the release or availability of core materials and separation of chemically reactive ingredients within a tablet or powder mixture. A wide variety of mechanisms is available to release encapsulated core materials; such as disruption of the coating can occur by ressure, shear or abrasion forces, permeability changes brought about enzymatically etc., improved gastro tolerability of drugs can be obtained by Microsphere.

D. Physical character of the final product

Microcapsules should have desirable physical properties like ability to flow, to be compacted or to be suspended and the capsule wall must be capable of resisting the pressure during compression etc.

TABLE-1 TYPES OF COATING MATERIALS USED TO PRODUCE MICROSPHERE	
Class of coating material	**Examples**
Gums	Gum Arabica, Agar, Sodium alginate, Carageenan.
Carbohydrates	Starch, Agarose, Sucrose, Dextran, Corn syrup, Chitoson.
Cellulose	Carboxy methyl cellulose, Methyl cellulose, Ethyl cellulose, Nitro cellulose, Acetyl cellulose, Cellulose acetate phthalate.
Lipids	Wax, Paraffin, Stearic acid, Bees wax, Oils, Fats, Hardened oil, Diglycerides, Monoglycerides.
Inorganic materials	Cellulose phosphate, Silicates, Clays. Proteins Albumins, Gelatin, Collagen, Casein, Gluten.
Chemically modified carbohydrate	Poly (acryl) Dextran, Poly (acryl) starch.
Non-biodegradable	AEROLEIN, Glycidyl methacrylate, Epoxy polymers.
Bio-degradable	Poly anhydrates, Poly alkyl cyno acrylate, Lactides and glycosides and their co-polymers.

Application:

1. Microspheres as drug delivery system or protein and peptides.
2. Microspheres as drug delivery system in cancer therapy.
3. Microspheres as local delivery system.
4. Microspheres for targeting of drug.
5. Microspheres in oral drug delivery.
6. Sustained drug delivery to brain.
7. Drug delivery to thee long.

Methods of Preparation

1. Solvent removed technique,

(A)Emulsion – solvent evaporation technique.

i) Oil in water (o/w) emulsion solvent evaporation

ii) Water in oil (w/o) emulation solvent evaporation

iii) Water in oil in water oil water (W/O/W)

emulsion solvent evaporation

(B)Emulsion solvent extraction.

(C)Emulsion solvent diffusion.

2. Coacervation and phase separation technique

(A)By temperature change.

(B)By incompatible polymer addition.

(C)By non solvent addition

(D)By salt addition

(E)By polymer polymer interaction

(F)By solvent evaporation

3. Cross – linking technique.

(A)Chemical cross linking.

(B)Thermal cross linking.

4. Polymerization Technique.

(A)Normal polymerization.

(B)Vinyl polymerization.

(C)Interfacial polymerization.

5. Spray drying and spray congealing

6. Freeze drying technique.

7. Precipitation technique.

8. Multi orifice centrifugal process

9. Pan coating.

10.Air suspension coating.

11.Melt dispersion technique.

1. Solvent Removed Technique

(A) Emulsion Solvent evaporation technique

This technique is based on the evaporation of the internal phase of an emulsion by agitation.

O/W Emulsification:

This technique is particularly suitable for encapsulation of lipophilic active principles such as steroid hormone, anti-inflammatory and neuroleptics.

The physicochemical properties of the active principle such as partition coefficient, its degree of ionization or its surfactant character play an important role in its localization.

W/O Emulsification:

E.g: Polymeric microspheres for drug delivery to oral cavity

Aqueous polymeric solutions were prepared and they were added in a dropwise manner to mineral oil containing 1% SPAN80 with continuous stirring.

The temperature was maintained at 60C for 24hr. allow the evaporation of dispersed aqueous phase.

The solied polymeric microparticles that separated from the oil on centrifugation were washed in hexane and dried in a vaccum dessicator.

iii)W/O/W Emulsification:

Active principles to be encapsulated are incorporated in an aqueous solution, which is poured into the casting organic solution of the polymer to form the emulsion of type W/O.

The primary emulsion is itself emulsified in external aqueous compartments to multiple emulsion of the type (W/O/W)

The organic phase acts as barrier between the two aqueous compartments preventing diffusion of drug towards the external aqueous phase.

In some cases gelatine/pectine/agarose are added into aqueous solution of active principle in order to hold the active principle in the aqueous internal phase by increasing the viscosity of internal phase.

E.g. Ethyl cellulose microspheres containing diclofenac sodium were prepared by this method.

Diclofenac sodium and gelatine were dissolved in water. The emulsion obtained was poured in solution of PVA (Polyvinyl Alcohol) in water to make a w/o/w emulsion and continuosly stirred. Microspheres were obtained.

(B)Emulsion Solvent Extraction Technique

In the emulsification-evaporation method, the organic solvent of dispered phase of the emulsion is eliminated in two stages

Diffusion of the solvent in the continuous phase (Solvent extraction) Elimination of the solvent at the continuous phase interphase.

In theory, if one uses a continuous phase, which will immediately extract the solvent of the dispersed phase, the evaporation stage is no longer necessary in formulation of microspherers. This can be achieved by Using large volume of continuous phase with respect to the dispersed phase. (Eg. 5flouro uracil biodegradable micropheres.) By using dispersed phase consisting of cosolvents, of which at least one has a great affinity for the continuous phase. One may even formulate a continuous phase with two solvents in which one act as solvents extractor of the dispersed phase (Eg. PLG microspheres containing diphtheria toxoid)

(C) Emulsion solvent diffusion :

The organic solution of drug and polymer is dispersed into aqueous medium with constant stirring. During the process solvent first diffuses out and then evaporating from the coacervate into the aqueous medium and forms the microspheres. As compared to emulsion – solvent evaporation this method is simple, quicker and does not required heat or any other harmful solvents.

2.Coacervation and Phase Separation Technique

The process is similar as for the preparation of microspheres. Microsphere by coacervation phase separation is generally attributed to the national cash

register (NCR) corporation and the patents of Green et.al. The general outline of the processes consists of three steps carried out under continuous agitation.

1. Formation of three immiscible chemical phases.
2. Disposition of the coating, and
3. Rigidization of the coating

a. By thermal change:

Phase separation of the dissolved polymer occurs in the form of immiscible liquid droplets, and if a core material is present in the system, under proper polymer concentration, temperature and agitation conditions, the liquid polymer droplets coalesce around the dispersed core material particles, thus forming the embryonic microcapsules. As the temperature decreases, one phase becomes polymer-poor (the Microsphere vehicle phase) and the second phase. (the coating material phase) becomes polymer-rich.

b. By incompatible polymer addition:

It involves liquid phase separation of a polymers coating material and Microsphere can be accomplished by utilizing the incompatibility of dissimilar polymers existing in a common solvent.

c. By non-solvent addition:

A liquid that is a non-solvent for a given polymer can be added to a solution of the polymer to induce phase separation. The resulting immiscible liquid polymer can be utilized to effect Microsphere of an immiscible core material.

d. By salt addition:

There are two types of coacervation: simple and complex. Simple coacervation involves the use of only one colloid, e.g. gelatin in water, and involves removal of the associated water from around the dispersed colloid by agents with a greater affinity for water, such as various alcohols and salts. The

dehydrated molecules of polymer tend to aggregate with surrounding molecules to form the coacervate. Complex coacervation involves the use of more than one colloid. Gelatin and acacia in water are most frequently used, and the coacervation is accomplished mainly by charge neutralization of the colloids carrying opposite charges rather than by dehydration.

e. By polymer-polymer interaction:

The interaction of oppositely charged poly electrolytes can result in the formation of a complex having such reduce solubility that phase separation occurs.

f. By solvent evaporation:

The processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent, which is dispersed in volatile solvents, which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation, the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer. In the case in which the core material is dissolved in the coating polymer solution, matrix type microcapsules are formed. The solvent evaporation technique to product microcapsules is applicable to a wide variety of core materials. The core materials may be either water soluble or water insoluble materials.

3. Cross Linking Technique

By Chemical and Thermal Cross Linking Microspheres made from natural polymers are made by cross linking process. This polymer includes Gelatin, Albumin, Starch,

Dextran In thos technique w/o emulsion is prepared, where Water phase is a solution of polymer containing the drug to be incorporated. Oily phase is a suitable vegetable oil or oil-mixture containing an oil soluble emulsifier. Once the desired emulsion is formed, the water-soluble polymer is solidified by some kind of

cross-linking process. This may involve thermal treatment or addition of a cross-linking agent such as glutaraldehyde to form a stable chemical cross-link as in albumin. Eg: Albumin, Chitosan beads prepared by heat stabilization method. Theophylline was used as drug. Chemical interactions between the drug, albumin and chitosan were evaluated using IR and HPLC. It was found that there was no intervention between them. Microspheres with different drug ratios were prepared and thermally cross-linked. Invitro release studies were carried out for a period of 8 hour and compared with pure drug. The microsphere containing 1:1:2 Drug: albumin: Chitosan ratio showed maximum release & highest drug entrapment.

4. Polymerization Technique

(A) Normal Polymerization:

The two processes are carried out in a liquied phase. Normal polymerization processed and carried out using different techniques as bulk, suspension, precipitation, emulsion and miceller polymerization processes.

i) In Bulk Polymerization, a monomer or a mixture of monomer along with the initiator is usually heated to initiate the polymerization and carry out the process. The catalyst or the initiator is added to the reaction mixture to facilitate or accelerate the rate of the reaction. The polymer so obtained may be moulded or fragmented as microspheres. For loading of drug, adsorptive drug loading or adding drug during the process of polymerization may be opted.

ii) The suspension polymerization, which is also referred to as the bead or pearl polymerization is carried out by heating the monomer or mixture of monomers with active principles(drugs) as droplets dispersion in a continuous aqueous phase.. The droplets may also contain an initiator and other additives.

iii) The emulsion polymerization differs from the suspension polymerization as due to presence of the initiator in the aqueous phase, which later on diffuses to the surface of the micelles or the emulsion globules. The bulk polymerization has an advantage of formation of the pure polymer, but it also suffers a disadvantage, as

it is very difficult to dissipate the heat of reaction, which can adversely affect the thermo labile active ingredients. On the other hand the suspension and emulsion polymerization can be carried out at lower temperature, since continuous external phases is normally water through which heat can easily dissipate. The two processes also lead to the formation of the higher molecular weight polymer at relatively faster rate. The major disadvantage of suspension and emulsion polymerization is, association of polymer with the unreacted monomer and other additives.

(B) Vinyl polymerization:

Vinyl monomers are polymerized. The microspheres are formed using techniques such as suspension, emulsion, soap less emulsion, dispersion and precipitation, seeding and support polymerization. Drug was incorporated within the monomers at the initial stage.

(C) Interfacial Polymerization:

Two complementary monomers are taken in a two-phase system and one of the phases is dispersed as droplets in another phase, drug is incorporated in any one phase and microsphere is obtained when condensation of monomers takes place at interface.

5. Spray Drying and Spray Congealing Method:

Nebulization or spray drying is widely used in the chemical, pharmaceauticaland food industries. The principle of spray drying rests on atomization of a solution by compressed air or nitrogen through a dessicating chamber and drying across a current if warm air.Laboratory spray dryer have

(1) Drying air inlet + filtration
(2) Heating
(3) Desiccation chamber
(4) Cyclone
(5) Collector for drying power microspheres

(6) Filtration + air outlet.

(7) Solution, suspension, emulsion to spray

(8) Compressed or nitrogen air

(9) Spray nozzle (e.g., pneumatic, ultrasonic)

Four separate phases may be distinguished

1. Nebulization of the solution in the form of aerosol
2. Contact of the nebulized solution with the warm air
3. Drying of the aerosol
4. Separation of the diried product and air charged with the solvent Spray Congealing:

Microsphere by spray congealing can be accomplished with spray drying equipment when the protective coating is applied as a melt. General process variables and conditions are quite similar to those already described, except thet the core material is dispersed in a coating material melt rather than a coating solution. Coating solidification (and Microsphere) is accomplished by spraying the hot mixture into a cool air stream. Waxes, fatty acids and alcohols, polymers and sugers, which are solids at room temperature but melt at reasonable temperature, are applicable to spray congealing techniques.

Congealable Dispersed Phase Technique:

A heated aqueous drug solution can be dispersed in molten wax to form a w/o emulsion which is emulsified in a heated external aqueous phase to form a w/o/w emulsion. The system is cooled and the microspheres collected. Camuba wax and beeswax can be used as coating materials. These wax-coated microspheres have been successfully tableted. Another alternative is to rapidly reduce the temperature when the primary emulsion is placed in the external aqueous phase.

6. Freeze Drying:

The freezing of the emulsion is done, the relative freezing points of continuous and dispersed phases are important. The continuous phase solvent is usually organic and is removed by sublimation at low temperature and pressure.

7. Precipitation Technique

The emulsion consists of polar droplets dispersed in non-polar medium.Solvent may be removed from the droplets by use of a cosolvent. The resultant increase in the polymer drug concentration causes a precipitation forming a suspension. Microsphere by Precipitation / Gelation of Na-alginate

Eg: Glipizide microspheres with a coat consisting of alginate and a mucoadhessive polymer such as sodium carboxy-methylcellulose, methylcellulose, carbopol and hydroxyl propyl-methylcellulose, were prepared by an orifice-ionic gelation process. Glipizide release from alginate-carbopol mucoadhessive microspheres was slow and extended over long period(14hr.) of time and depended on the composition of coat of the microspheres.

8. Multiorifice – centrifugal process

The South-West research institute (SWRI) has developed a mechanical process for producing microcapsules that utilizes centrifugal forces to hurl, a core material particle through an enveloping Microsphere membrane therapy effecting mechanical Microsphere. Processing variables include the rotational speed of the cylinder, the flow rate of the core and coating materials, the concentration and viscosity of the coating material and the viscosity and surface tension of the core material. This method is capable of microencapsulating liquids and solids of varied size ranges, with diverse coating materials.

9. Pan coating:

The microcapsulation of relatively large particles by pan coating method has become wide spread in the pharmaceutical industry and solid particles greater

than 600 µg in size are generally considered essential for effective coating. The coating is applied as a solution or as an automized spray to the desired solid core passed over the coated materials during coatings are being applied in the coating pans.

10. Air-suspension coating:

Air-suspension coating of particles by solutions or melts gives better control and flexibility. The particles are coated while suspended in an upward-moving air stream. They are supported by a perforated plate having different patterns of holes inside and outside a cylindrical insert. Just sufficient air is permitted to rise through the outer annular space to fluidize the settling particles. Most of the rising air (usually heated) flows inside the cylinder, causing the particles to rise rapidly. At the top, as the air stream diverges and slows, they settle back onto the outer bed and move downward to repeat the cycle. The particles pass through the inner cylinder many times in a few minutes.

11. Melt-dispersion technique:

In this technique the coating material is melted by heating upto 80oC. The drug is suspended in it and then emulsified in water containing emulsifying agent at 80oC under stirring. Microcapsules are formed as the temperature of the system reaches to room temperature.

Evaluation of Microspheres:

The characterization of microcapsule carrier is an important phenomenon, which helps to design a suitable carrier for the proteins, drug or antigen delivery. The parameters that are generally evaluated for characterization of microcapsules are:

1. Microsphere morphology
2. Microsphere size and shape
3. Drug content or drug loading
4. Mass loss and degree of hydration

5. In-Vitro drug release study
6. Density determination.
7. Iso electric point.
8. Capture efficiency.
9. Contact angle.

1. Microsphere morphology:

The internal and external morphologies of microspheres were visualized using SEM (Scanning electron Microscopy), Electron microscopy, and Scanning tunneling microscopy. Fourier transforms Raman spectroscopy or X-ray photoelectron spectroscopy may be used to determine if any of the material which should have been entrapped is present on the surface and if any other contaminates are present. Other surface characterization techniques include surface charge analysis using microelectronphoresis.Surface charge can provide information regarding microspheres aggregation. Surface charge is an important parameter with respect to the interaction of microspheres within the body. Following i.v Injection, microspheres can be taken by the macrophage or monocyte cells present in the plasma; surface charge is one of the parameters, which determines whether this takes place. Surface forces are important in the entrapment, wetting and adhesion of core material by the coating material. The wettability of solids by different liquids is assessed by contact angle measurements. When wetting of core material is poor, it is difficult or impossible to form microspheres

.***2. Microsphere size***

The most widely used procedure to visualize microcapsule are conventional light microscopy, and Scanning electron microscopy (SEM). Both techniques can be used to determine the shape and outer structure of microcapsule. SEM provides higher resolution in contrast to the light microscopy. It allows investigation of the microsphere surfaces and after particles are cross sectioned, it can also be

used for the investigation of double walled systems. Confocal laser scanning microscopy (CLSM) is applied as a nondestructive visualization technique, which allows characterization of structures not only on surface, but also inside particle. Different methods used are:

a. Light microscopy
b. Resistance blockage techniques (Coulter analysis)
c. Light blockage techniques
d. Light scattering
e. Laser diffraction analysis

Microscopic method For particles less than 1μm, photon correlation spectroscopy is used. Average particle size is calculated from SEM photomicrographs. The size distribution can be carried out by optical microscopy, or using a Beckman-Coulter counter multisizer. A 100 micrometer orifice is generally used and at least 50000 particles were counted for each sample. The particle size distribution of the microspheres is also determined by laser scattering instrument.

3. Drug loading or drug content

Differential scanning calorimetric and U.V. Spectrophotometric methods were employed to verify the presence of drug in microspheres and its chemical stability. The differential scanning calorimeter was used to obtain the thermograms of various formulations.

Encapsulation Efficiency is defined as: E% = (La/Lt)*100

La = actual loading

Lt = Theoretical loading

4. Mass loss and degree of hydration:

The wet microspheres were weighed accurately (Wet wt. = Ww) dried for 48 hrs under reduced pressures at room temperature and weighted again. (dry wt. = Wd)

MR (%) = (Wd/Ww)*100

DH = (Ww – Wd) Wd

Where,

MR = Mass remaining,
DH = Degree of hydration,
Wd = dry weight,
Ww = Initial wt of microspheres

5. In vitro drug release

To find out the in vitro release the most commonly used techniques is as follows: In vitro release profile of drug from microspheres is examined in Phosphate buffer of pH 7.4 from 3-10 hr using the rotating basket method specified in USP XX1 AT 100 rpm. Microspheres equivalent to 50mg of drug were suspended in the dissolution medium and the medium was maintained at 37°C , 5 ml of samples were withdrawn periodically at intervals of half an hour ad same volume of fresh medium was replaced in to the breaker. The concentration of drug released at different time intervals was then determined by measuring the absorbance using spectrophotometer.

6. Density determination

The density of the microcapsule can be measured by using a multi volume pychnometer. Accurately weighed sample in a cup is placed in pychnometer, helium is introduced at a constant pressure in chamber and allowed to expand. The expansion results in a decrease in pressure within the chamber. From two pressure readings the volume and hence density of microcapsule can be determined.

7. Isoelectric point:

The micro electrophoresis is an apparatus used to measure electrophoretic mobility of microsphere from which the isoelectric point can be determined. The

electrophoretic mobility can be related to surface contained charge, ionisable behavior or ion absorption nature of microsphere.

8. Capture efficiency

The capture efficiency of microcapsule or the percent drug entrapment can be determined by allowing washed microcapsule to lyse. The lysate is then subjected to determination of active constituents as per monograph. The percent encapsulation efficiency is calculated using following equation

% Entrapment = actual content/theoretical content x 100

9. Contact angle

The angle of contact is measured to determine the wetting property of microcapsule. It determines the nature of microsphere in terms of hydrophilicity or hydrophobicity. The angle of contact is measured at the solid/air/water surface by placing a droplet in circular cell mounted above the objective of inverted microscope. Contact angle is measured at 20oC within a minute of decomposition of microspheres.

CHAPTER – III

LITERATURE REVIEW

LITERATURE REVIEW

[H. Abdul Rahman et al., 2017] prepared chitosan microspheres for the delivery of chemotherapeutic agents, using paclitaxel as a model. Chitosan had unique physiochemical and biological features that suggested as a good candidate for the development of safe and effective drug delivery systems. The development of formulations of targeted delivery systems for the chemotherapeutic agents like paclitaxel can potentially alleviate the systemic cytotoxicity as well as directing therapy to the specific lesions. The main aim of this work was to critically evaluate the use of chitosan microspheres as a drug delivery system to enhance paclitaxel distribution and efficacy in specific targeted sites. The medium molecular weight of chitosan microspheres exhibited the highest paclitaxel dissolution rate because of the highest aqueous perfusable microspheres, where 90% of the drug could be released from the formulation and p^H of the formulation and it was between 21-83.7% then the cumulative release amount was increased within 24hours.

[Banasmita K., et al., 2017] formulated and evaluated metronidazole microspheres-loaded bioadhesive vaginal gel to ensure longer residence time at the infection site, providing a favourable release profile for the drug. Microspheres were prepared by solvent evaporation method in various ratios of metronidazole to poly-ε-caprolactone(PCL). The optimized batch of microspheres F4 showed entrapment efficiency of 72.62%, solubility 1.5mg/ml, and partition coefficient of 0.12. Particle size of all the formulations was observed below 100µm. Regular and spherical particles were observed in the SEM photomicrographs. The optimized gel formulation G5 showed viscosity of 7538cps at 100 rpm, gel strength recorded as 35 secobds for a 1000 mg load and spreadability of 4.6 g.cm/seconds. G5 showed 82.4%drug release at 10 hours and mucoadhesive strength of 6.5±1.2 g. The study results suggested that metronidazole-loaded PCL microspheres in mucoadhesive gel would provide a mean for sustained treatment of vaginal infections.

[Daniel G et al., 2017] designed and developed a novel sitagliptin-loaded transdermal patch for diabetes treatment. Sitagliptin was formulated in a gel-type reservoir on a transdermal patch device, optimized by a mathematical simulation methodology, and experimentally validated in vitro using a franz cell apparatus. The mathematical model determined optimal design parameters which included 1% w/w acellulose as drug reservoir, transdermal patch rate control membranes, 1.25 mM initial drug concentration, 2 mL initial volume, and 4.52 cm^2 patch area. The optimized reservoir formulation was then fabricated in the transdermal patch system and tested using Franz cell to validate the simulation. The experimental results from the fabricated transdermal patch system indicated that sitagliptin can be formulated in a patch to achieve the target effective plasma drug concentration in less than one hour and is able to sustain glucose control for over 24 hours.

[Bhagat BV., et al., 2016] formulated and evaluated the rifampicin loaded microspheres by non-aqueous solvent evaporation method. Ethyl cellulose and carboxy methyl cellulose were used as the retardant materials. The prepared microspheres were red, free flowing and spherical in shape. It was observed that the increase in concentration of the polymer, increases the mean particle size of the microspheres. The mean particle size of the prepared microspheres was found to be 40.14 ± 1.17 to 53.88 ± 2.1µm. The maximum yield of the microspheres was found to be 87.27% and the encapsulation efficiency was found to be 27%. In-vitro release studies indicated that the formulation F1 released 80.567% of rifampicin at the end of 12 hours. Thus the prepared microspheres proved to be a potential candidate as a microparticulate drug delivery in this area of patent novel.

[Hitesh K., et al., 2016] fabricated and characterized metformin hydrochloride floating microspheres using Eudragit RS 100 as a retardant polymer, by non-aqueous solvent evaporation method. The influence of formulation factors on particle size, encapsulation efficiency and in vitro release characteristics of the microspheres were investigated. SEM showed that the

microspheres were spherical and smooth in texture. Drug entrapment efficiency was found to increase with increase in polymer concentration. At 3:1 polymer: drug ratio ES3, drug entrapment efficiency was found to be maximum 79.12%, buoyancy of optimized formulation ES3 found to be 95% and they were in the size range of 200-300 μm by all formulation. The in-vitro drug release profile was biphasic with an initial burst release 19.23% in 1hour attributed to surface associated drug, followed by a slower release phase, 83.27% drug release after 12 hours there was a sustained release of a drug at a constant rate.

[Ramya B and Krishna A., 2015]prepared and evaluated floating microspheres of omeprazole by solvent evaporation method. Process parameters such as stirring speed, stirring time and organic to aqueous phase ratio were optimized. Floating microspheres were prepared by non-effervescent system. Ethyl cellulose and Eudragit S 100 used as a swollen polymers. F4 formulation showing highest entrapment efficiency, was found to be 96.8%, drug content was 60.9%. In a time period of 12 hours 70.3% of drug had been released from F10 formulation. The percentage of buoyancy was found to be 70.3%. From the results , 1:30 drug to polymer ratio at 1:5 organic to aqueous phase ratio omeprazole could be formulated with good entrapment efficiency, sustained release property and highest percentage of buoyancy.

[Irin D., et al.,2015] formulated and evaluated glibenclamide microspheres using different polymers such as ethyl cellulose, eudragit RS/RL 100 and methocel K15 by non-aqueous solvent evaporation technique. The maximum and minimum release of drug was observed 90.99% and 71.98%. Percent of actual drug entrapment varied from 7.89% to 15.36% and percent of drug entrapment efficiency varied from 69.23% to 98.21%. The in-vitro studies showed that after the end of 8 hours of dissolution, the release drug from microspheres was 98.21%.

[Prasanthi S., et al., 2015] formulated and evaluated sitagliptin phosphate and simvastatin bilayered tablets by direct compression technique. Ten formulations of sitagliptin phosphate and simvastatin bilayered tablets were prepared by varying the ratios of polymers in the sitagliptin phosphate layer and simvastatin layer F1 to F10 by direct compression technique. All the powdered blends of formulations were evaluated for pre-compression parameters for flow properties such as angle of repose, tapped density, compressibility index and post compression parameters such as thickness, weight variation, friability, hardness, drug content, in-vitro disintegration and dissolution studies and they were found to be within USP limits. Among all formulations F7 was optimized as best formulation. F7 formulaltion showed 97.23% for sitagliptin phosphate and simvastatin 98.32%, maximum drug release at the end of 45 minutes.

[Tanvi P., et al.,2015] formulated and evaluated floating tablets of sitagliptin phosphate prepared by employing different polymers like guar gum, carbopol 940, and HPMC and magnesium stearate by effervescent technique. Sodium bicarbonate and citric acid were incorporated as gas generating agent. The tablets were produced by direct compression method. The floating tablets were evaluated for uniformity of weight, hardness, friability, drug content, swelling studies, dissolution studies, disintegration studies and stability studies. All the prepared batches showed good results of in-vitro studies of tablets. It was aimed to prepare for prolong residence in the stomach over conventional gastro-retentive approaches.

[Sunita Shakya., 2015] formulated and optimized immediate release tablet of sitagliptin phosphate using response surface methodology for the management of type-II diabetes mellitus. Different formulations of immediate release were prepared by applying 2 factor 2 level Central Composite Design using Minitab 16 which gave 13 formulations for each layer. The amount of Sodium Starch Glycollate(SSG) and croscarmellose sodium in IR layer were used as independent variables and the percent drug release at 15 minutes were selected as dependant variables for optimization. Based on the in-vitro dissolution data, the composition

of formulation with optimum drug release for immediate release were identified and employed to formulate optimized tablets followed by its evaluation. All the physico-chemical parameters of the tablets were found satisfactory. The optimized sitagliptin phosphate IR tablet disintegrated in 14 seconds and showed an initial release of sitagliptin 99.072% within 15 minutes.

[Alini D., et al., 2014] developed and validated the conditions for the dissolution method for coated tablets based on in-vivo data for improving medium sensitivity by investigating a possible in vivo- in vitro correlation. Several parameters were tested to develop the method, and the following conditions were considered satisfactory: p^H 6.8 phosphate buffer, 900ml of dissolution medium, temperature at 37 ± 1°c, paddle apparatus, and rotation speed at 50 rpm. The dissolved percentage of sitagliptin was quantified by high performance liquid chromatography. The method is linear in the range of 10.0-70.0 µg/ml, precise, with RSD values less than 2%, and accurate. The dissolution method as developed and validated supplied a good IVIVC when employing p^H 6.8 phosphate buffer medium, which can be used in quality control of sitagliptin coated tablets since no official method has been described.

[Rashmi R., et al., 2014] developed and evaluated sustained release microspheres of glibenclamide using ethyl cellulose N 100 as a rate retardant polymer, by emulsion solvent evaporation method. SEM studies showed that the microspheres were spherical and porous in nature, the mean particle size ranging from 111.3 µm to 140.12µm. The batch EC5 showed highest drug entrapment efficiency of about 82.33%. The in-vitro release studies revealed that the drug release was sustained up to 24 hours. The in-vivo studies were performed in healthy rabbits to analyze the floating efficiency of microspheres. Microspheres of glibenclamide were prepared successfully and could help to manage better the complications involved in type II diabetes.

[Pavan Kumar and Sree Priya., et al., 2014] formulated and evaluated floating microspheres of dextromethorphan hydrobromide by solvent evaporation technique using polymers like ethyl cellulose and hydroxyl propyl methyl cellulose in different ratios. The increased concentration of polymer affected the particle size, percentage yield, in-vitro buoyancy and drug release from the microspheres. Percentage yield of microspheres was found up to 71.16%. The formulated microspheres were free flowing with good flow properties. SEM of optimized formulation F3 confirmed spherical structure of microspheres with pores on the rough surface and the particles were of the size range from 112-224 μm. The floating microspheres of optimized formulation F3, exhibited the prolonged drug release of 99.61% in sustained manner up to 12 hours and remain buoyant more than 12 hours. The drug release mechanism from the floating microspheres was found to be anamolus type(non-fickian diffusion) and followed Higuchi kinetics.

[Ramesh Y., et al., 2013] formulated and evaluated floating microspheres of norfloxacin by non-aqueous solvent diffusion method using polymers hydroxyl propyl methyl cellulose and ethyl cellulose. The SEM photographs of the microspheres revealed that the microspheres were spherical with smooth surface and slightly aggregated and size range was 340-425 μm. The formulation NF-XIII showed highest percentage yield 98.5%, and highest entrapment efficiency 99%. The buoyancy results indicated that all formulations floated for more than 12 hours over the surface of the dissolution medium without any apparent gelation. The in-vitro drug release revealed that batch NF-XIII was having 98.8% cumulative release at the end of 10th hour when compared with all batches due to increase in polymer concentration as seen in all formulation.

[Saudagar R B et al., 2013] formulated and evaluated sustained release microspheres of Ropinirole hydrochloride using ethyl cellulose by w/o type emulsion solvent evaporation technique and bees wax based microspheres was prepared by spray drying technique. Thus the sustained release microsphere of ropinirole hydrochloride for 12 hours were successfullu designed, optimized and characterized by using potential polymers of ethyl cellulose and beeswax.

[Navneet Garud and Akanksha., 2012] formulated metformin microspheres by non-aqueous solvent evaporation method using various polymers including ethyl cellulose, hydroxyl propyl methyl cellulose, carbopol 934P and chitosan. The effect of process variables, viz, drug/polymer ratio, stirring rate and type of polymer on the mean particle size, and drug release of the microspheres were studied. It was observed that as the stirring speed increased from 600 to 1800 rpm, microsphere size decreased and hence drug release rate increased. Drug release rate at 1:2 drug: polymer for microspheres produced at a stirring rate of 1200 rpm was in the following order: carbopol 934P>HPMC>ethyl cellulose>chitosan. The formulations containing carbopol 934P and HPMC released drug faster than chitosan microspheres, Amongst the developed microspheres, CH3 formulation exhibited maximum prolonged drug release at gastrointestinal p^H or at least 15 hours. This oral sustained metformin formulation could potentially improve the bioavailability of the drug as well as patient compliance.

[Manish D., et al., 2012] formulated and evaluated floating microspheres of metformin hydrochloride using hydroxyl propyl methyl cellulose and Eudragit RS 100 polymers by emulsion solvent evaporation technique. The average particle size of microspheres were between 608 and 864 µm. The SEM studies showed microspheres were spherical, discrete and having a rough surface. The in-vitro drug release studies indicated that the percent drug release rate for F4 formulation showed high release than all formulations.

[Lydia F., et al., 2011] achieved controlled delivery of Gentamicin using poly(3-hydroxybutyrate) microspheres using a solid-in-oil-water (s/o/w) technique. For this study, several parameters such as polymer concentration, surfactant, and stirring rates were varied in order to determine the effect on microsphere characteristics. The average size of the microspheres was in the range of 2µm to 1.54µm, low stirring speed of 300rpm produced slightly larger microspheres when compared to the smaller microspheres produced when the stirring velocity was increased to 800rpm. The surface morphology of the microspheres appeared

smooth when observed under SEM. The microspheres exhibiting the highest encapsulation efficiency was 48%. The in-vitro release of gentamicin was bimodal, an initial burst release was observed followed by a diffusion mediated sustained release, finally after 20 hours, the cumulative drug release was 95.33%.

[Saritha D., et al., 2011] formulated and evaluated ramipril microspheres produced by the non-aqueous emulsification solvent evaporation method. The microspheres were prepared using polymethacrylate polymers and hypermellose. The impact of different factors such as stirring rate, polymer concentration, and volume at processing medium on the characteristics of the microspheres were investigated. Surface of the microspheres was investigated by SEM, showed they were spherical in shape and exhibited porous surface it suggested that the drug was released through pores and the mechanism of drug release was diffusion controlled. The mean particle size of the formulation F1 to F4 was found to be increased from 52 μm ± 1.5 to 100 μm ± 0.52 due to increasing polymer concentration. The entrapment efficiency was found to be increase from 52.43% ±0.45 to 23.19% ± 0.66 this could be due to the increased viscosity in a fixed volume of solvent as the drug to polymer ratio increased. In-vitro release studies were showed the increase in drug release rate from 20th & 18th hours was observed due to higher migration of drug to the surface, increasing stirring speed, decreased particle size and increased larger available surface area. Eudragit RL 100 polymer increased the porosity of the matrix and they accelerated the drug release.

[Sudhamani . et al.,2011] prepared and evaluated of ethyl cellulose microspheres of ibuprofen for sustained drug delivery. These ethyl cellulose microspheres were prepared by the solvent evaporation method. The prepared microspheres were subjected to various evaluation and in-vitro release studies. Highest percentage of loading was obtained by increasing the amount of ibuprofen with respect to polymer. The particle sizes of the prepared microspheres were determined.

[Kumar,Ankit .et al.,2011] evaluated microencapsulation this approach facilitates accurate delivery of small quantities of potent drugs, reduced drug concentrations at sites other than the target organ or tissue and protection of labile compounds before and after administration and prior to appearance at the site of action. Microencapsulation system offers potential advantages over conventional drug delivery systems. Microspheres and microparticales are a unique carrier system for various pharmaceuticals dosage form.

[Vijaya Ramesh et al.,2011] used different polymers for the development of microparticles for controlled release of antibiotic drug ciprofloxacin. Microspheres were prepared by emulsion solvent evaporation technique. Attempts are also made to increase the entrapment efficiency by changing experimental variables.

[Chinna B., et al., 2010] formulated and evaluated indomethacin microspheres using natural and synthetic polymer such as egg albumin, ethyl cellulose, eudragit L 100 as the retardant materials for controlled release preparation of a highly water- soluble drug, indomethacin. Microspheres were prepared by solvent evaporation method using an acetone/liquid paraffin system and phase separation co-acervation method. The prepared microspheres were evaluated and characterized, the mean particle size of the microspheres was found in the range of 150 to 400μm. The drug-loaded microspheres showed 70-86% of entrapment and release was extended up to 6 to 8 hours releasing 86% the total drug from the microspheres. The release of indomethacin was influenced by the drug to polymer ratio and particle size was found to be both diffusion and dissolution controlled.

[Subbiah G., et al., 2010] formulated and evaluated controlled release formulation of idarubicin microspheres using biodegradable hydrophilic and hydrophobic polymer mixtures such as ethyl cellulose and HPMC by using solvent evaporation method. By increasing the concentration of ethyl cellulose the mean particle size also increased , with change in the speed of rotation, there is an influence on the size of the microspheres that varied with increase in size with

decrease in rotation and was in the range between 134.0±9.0 to 424.7±11.2 μm for 1000rpm and 500rpm respectively. But the entrapment capacity did not show much significant change in change in speed of rotation falling between 54% and 62%. The release of idarubicin microspheres showed Higuchi's square root model. This model showed that the idarubicin can be attempted to maintain sustained release and reducing the side effects produced by the drug.

[Phutane P., et al., 2010] formulated and evaluated novel sustained release microspheres of glipizide using Eudragit RS 100 and ethyl cellulose as a rate retardant polymers by the emulsion solvent diffusion-evaporation technique by using the modified ethanol,-dichloromethane co-solvent system. SEM indicated that the microspheres were spherical with smooth surface and not aggregated. Maximum encapsulation efficiency was observed of the batch F3 was 94.84%. The combination of Eudragit RS 100 and ethyl cellulose had been achieved in batch F3 where extended release of drug for approximately 12 hours had been obtained and the %drug release at 12th hour was 96.76%

[YeM. et al.,2010] shown that biodegradable microparticles can be applied in long-term protein delivery. The conventional way of delivering a protein drug needs daily, sometimes multiple, injections to achieve its therapeutic effectiveness. To perfect patient compliance and ease, sustained release dosage forms have been developed.This section examines the properties of protein-loaded microparticles, in specific, protein loading and release characteristics from PLGA microparticles.

[Shashank tiwari. et al.,2010] formulated micoencapsulation by solvent evaporation method (study of effect process variables) the properties of poly (lacticacid) (poly lactic co glycolic acid) the effect of this microencapsulation is successful .and to get a desire release rate and highest drug loading.

[Anand,Chirag. et al.,2010] formulated and evaluated of floating micropsheres of captopril for prolong gastric residence time. Floating microspheres was formulated using biocompatible polymers like Eudragit S100

and Ethyl cellulose in different proportions by solvent evaporation technique. The prepared microspheres were evaluated for percentage yield, micromeritic properties, particle size, morphology, drug entrapment, buoyancy studies, In vitro drug release studies, practical yield of the microspheres.

[Umamaheshwari. et al.,2010] developed effective drug delivery systems for the treatment of Helicobacter pylori infection using polycarbonate (PC) floating microspheres as drug carriers. In an effort to augment the anti-H. pylori effect of acetohydroxamic acid (AHA), floating PC microspheres, which have the ability to reside in the gastrointestinal (GI) tract for an extended period, were prepared by emulsion (O/W) solvent evaporation technique. The effect of PC concentration on the morphology, particle size, entrapment efficiency and drug release rate was studied. Invitro studies confirmed the excellent floating properties of PC microspheres. In conclusion, the floating microspheres more effectively cleared H. pylori from the GI tract than the drug because of the prolonged gastric residence time resulting from the excellent buoyancy of the PC.

[Kannan k., et al., 2009] formulated and evaluated sustained release microspheres of acetazolamide by solvent evaporation technique using Eudragit RL/RS as a rate retardant polymer. Spherical particles were obtained, when the polymer and drug ratio was increased (2:1, 3:1) at stirring speed 750 rpm. The average size of the microspheres was found as 250μm in all the formulations. The in-vitro release of the drug from microspheres showed that the release rates were very slow in Eudragit RS and in the release rate is fast in the microspheres made by Eudragit RL. So the combination of the polymers Eudragit RS and Eudragit RL gives desired release of drug from the microspheres.

[Durgacharan A., et al., 2009] formulated and evaluated controlled release microspheres of isosorbide dinitrate by non-aqueous emulsification solvent evaporation method using acrycoat S 100 as matrix polymer. The mean particle size of the microspheres was in the range 140.2 ±8.3 to 248.7 ± 17.24 μm. SEM showed that they were spherical with a smooth surface and exhibited a range of sizes within each batch. The incorporation efficiency was showed higher at

89.24% ± 2.1 in A3 batch. The in-vitro release studies were performed for 12 hours and the cumulative release of isosorbide dinitrate significantly decreased with increasing acrycoat S 100 polymer concentration. Smaller microspheres were formed at lower polymer concentration and had a larger surface area exposed to dissolution medium, release was higher in the case of microspheres prepared at a higher agitation speed but at low agitation speed the release rate was slow.

[Vadhana singh et al.,2009] Develop the hollow microspheres as a new dosage form of floating drug delivery system with prolonged stomach retention time. Hollow microspheres containing ranitidine hydrochloride were prepared by solvent evaporation method using Eudragit RLPO dissolved in a mixture of dichloromethane and ethanol. Hollow microspheres could prolong drug release time (approximately 24 hrs) and float over stimulate gastric fluid for more than 12hrs.

[Kem et al.,2009] produced uniform microspheres and double-wall microspheres capable of efficiently encapsulating model drugs. Of primary importance was the ability of monodisperse microsphere formulations. Monodisperse PPF microspheres and core-shell microparticles offer advantages in reproducibility, control, and consistency that may provide valuable assistance in designing advanced drug delivery systems to achieve precise control of the particle size and reproducibly fabricate nanocapsules the technology.

[Om praksh et al., 2009] Evaluated rosin polymer, Rosin application agent, microencapsulating agent. It had been found anti-inflammatory and antitumor activity. Its semisolid preparation such as skin cream shows good homogeneity and spreadibility. It has prominent property for the sustained release drug system with most of the drug and dosage form.

[Alaghusundram., et al.,2009] reported microspheres not only for prolonged release, but also for targeting of anticancer drugs to the tumour. In future by combining various other strategies, Microspheres will find the central

place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective in-vivo.

[Amal H. et al.,2009] formulated and evaluated captopril Sustained release microparticles.using acetate propionate and employing the solvent evaporation technique .it decreases the side effects.microparticles containing different dug and polymer ratio.

[Naikwade S et al.,2009] studied the pulmonary delivery of budesonide microparticles formulation and in vitro determination by spray drying. Prepared Microparticles were spherical in shape and they are characterized by smooth surface with low-density particles. Formulations shown extended in vitro drug release up to 4 hours thus use of microparticles possibility offers sustained release profile along with increase delivery of drug to the pulmonary tract.

[Roy S et al.,2009] prepared mefenamic acid microspheres by cross linking chitosan with gluteraldehyde. The invitro release pattern was found to follow zero order release as the dissolution exponent come nearer to 1.

[A. Dalmoro et al.,2009] prepared enteric microparticles for controlled and made drug delivery applications through different ways of microencapsulation (namely single emulsions: water in oil-W/O; oil in water-O/W; or double emulsions: water-in oil-in water-W/O/W) and their impact on final properties of the product. Microcapsules or microsphers can be designed to progressively release active ingredients. A coating may also be given to open in specific areas of body “smart polymers” which are perfect candidates for advancing self-regulated delivery systems.

[Ravi Kumar Reddy J. et al.,2009] investigated the delayed release microparticles prepared from different polymers by emulsion-solvent evaporation method and examined the physic-chemical characters. The mechanism of drug release was set up to be erosion as it was caused by (1-Mt/M) 1/3 versus time plots. Relative drug release study allow that the formulated product have more sustained effect than the marketed product.

[Vinay mishara et al.,2008] prepared and evaluated matrix microspheres system for simultaneous and sustained release of Candesartan cilexetil and captopril for the management of nephritic syndrome, Ethyl cellulose was used as a retardant polymer decrease of side effect, increase of bioavailability and therapeutic action of both combination drugs.

[KBR chowdary, Dana.,2008] reported that the study is to evaluate ethyl cellulose as a coat for controlled release microcapsules of diclofenac.. Ethyl cellulose coated microcapsules were prepared by an emulsion-solvent evaporation method employing different proportions of core and coat and the microcapsules were evaluated for size, drug content and microencapsulation efficiency.

[Rajashree hirlekar et al.,2008] developed muco-adhesive drug delivery system of Metoprolol tartarate using various polymer like sodium carboxy methyl cellulose and natural polymers like gum karaya, xanthum gum and locust bean gum. The formulation containing xanthum gum and locust bean gum in 2:1 ratio exhibited complete drug release in 45 minutes.

[Lu et a.,2008] formulated a tumor-Penetrating microparticles for intraperitoneal therapy of Ovarian Cancer using PLG copolymers. These tumor-penetrating microparticles were prepared by Emulsion–solvent evaporation method. The present study provided several findings that may be applied to improving intraperitoneal therapy.

[Dr.lakshmanu prabu, shirwaikar.AR et al, 2007] prepared the sustained release Aceclofenac microspheres by using rosin as an encapsulating polymer. The release rate of drug from the microspheres could be properly controlled for about 24h. Appropriate variation in the proportions of drug; polymer and stabilizer can lead to a product with the desired controlled release features.

[Juergen Siepmann et al.,2006] envisaged that microparticles offer an effectual defence of the encapsulated active agent against (e.g. enzymatic) degradation, (ii) the chance to precise control the release rate of the incorporated drug above periods of hours to months, and (iii) an easy administration.

[Kevin et al.,2006] emphasized that controlled release drug delivery systems are being evolved to address many of the difficulties connect with conventional methods of administration. Controlled release drug delivery utilize devices such as polymer-based disks, rods, pellets, or microparticles that incorporate drug and release it at controlled rates for comparatively long periods of time.

[El-Kamel et al., 2001] 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.

CHAPTER – IV

AIM OF WORK

AIM OF WORK

Sitagliptin phosphate is an insulin-sensitizing anti-diabetic drug. It was chosen as a model drug since it has a half-life (8-14 h) and bioavailability 87%. It belongs to BCS Class III drug(high solubility and low permeability). Low permeability leads to potential problem of poor bioavailability and bioequivalence of drug dosage form. Furthermore, micronized sitagliptin phosphate has shown better absorption than nonmicronized form. Hence, the absorption of drug can be enhanced using transdermal microsphere gel technique.

Sitagliptin phosphate is a dipeptidyl peptidase 4-(DPP-4) inhibitor with glucose control capabilities that was effectively used for treating diabetes in the past. However, the oral administration of this drug caused such severe side effects that it was removed from the market.

Sitagliptin is an ideal candidate in considering transdermal diabetic drug transport treatment due to its ability to effectively treat diabetes and small overall size. Past studies have concluded that sitagliptin does not cause hypoglycaemia, and it requires a low effective concentration in the body. The minimum effective concentration of sitagliptin required in the blood is 100 nM. When sitagliptin is taken orally, there are many negative side effects that have been reported which include severe digestive discomfort, general pain, upper respiratory tract infections, and headache. However, by delivering sitagliptin transdermally, there is potential for these side effects to be circumvented through avoiding digestive overload.

Type II diabetes is caused by insulin resistance and occurs when the pancreas produces insulin, though the body does not appropriately react to the protein. Type II diabetes increases one’s risk for many other health problems such as heart and blood vessel diseases, kidney damage, vision degeneration, nerve damage, and foot damage. Patients with type 2 diabetes continuously alternate between a hyperglycaemic state, which refers to high blood glucose and low blood

glucose levels, respectively. It is imperative for these patients to monitor their blood glucose levels in order to remain in good health.

Oral drug delivery and liquid injections are currently the two most common forms of diabetes treatment. However, both methods implicitly present limitaitons. Patients who take oral anti-diabetic medications typically require high dosages in order for sufficient efficacy due to low bioavailability in drug of this class. Patients also tend to experience negative side effects, such as vomiting and digestive pain from taking high dosage oral anti-diabetic medications. Patients who take hypodermic insulin injections are required to inject themselves with insulin up to three times a day; this method is very painful and inconvenient for patients who are dependant on this life-saving protein. A feasible treatment option for diabetes lays in transdermal drug delivery approaches. It has been shown that the skin is a barrier that can be exploited for drugs to enter the body, so transdermal patch or cream formulation has potential for development as an alternative to the typical diabetes treatment.

Furthermore, the use of transdermal patches can provide sustained drug release over hours, and even days. This novel methodology for diabetes treatment would have the ability to enable patients to lead quality lives without the pain from the numerous hypodermic insulin injections or the negative side effects caused by oral anti-diabetic medications. However, this delivery method possesses limitations of its own, such as the necessity of selecting a drug small enough to facilitate transdermal diffusion.

Microspheres have been widely accepted as a means to achieve sustained release drug delivery system. The microspheres requires a polymeric substance as a carrier and a core material. Among the various methods developed for formulation of microspheres, the non-aqueous solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of drug.

Eudragit RS 100 is referred as a ammoniomethacrylate copolymers, with the former having 5%functional quaternary ammonium groups and the latter having 10% functional quaternary ammonium groups. Eudragit RS 100 is a water-insoluble polymer that is widely used as a wall material for sustained release microspheres due to its biocompatibility, good stability, easy fabrication and low cost.

HPMC is a non-ionic, swellable polymer. Hydrophyllic polymer gel matrix systems are widely used in sustained drug delivery to obtain a desirable drug release profile and cost effectiveness because of their flexibility. The hydration rate of HPMC increases with increase in the hydroxyl propy content.

The use of natural polymers is preferred due to their proven biocompatibility and safety. In this respect, chitosan, a cationic polymer, has attracted particular attention. It is biocompatible and bioadhesive at physiological p^H ande possesses OH and NH_2 groups that can give rise to hydrogen bonding. It is a high molecular weight polysaccharide, compromising of glucosamine and N-acetyl glucosamine obtained by deacetylation of chitin. Because of its low production cost, non-toxic nature, chitosan has found application in multiparticulate drug delivery.

Carbopol is a polymer consisting of acrylic acid cross-linked with either polyalkenyl ether or divinyl glycol. It readily absorbs water, gets hydrated and swell. In addition to hydrophilic nature, cross-linked structure and insolubility in water, due to the high proportion of the carboxy groups present, carbomer solution is known to be acidic. It is also low viscosity but when neutralized with triethanolamine, it is converted to high viscous gels. The adhesive properties of carbomer are exploited to develop bioadhesive gels and drug delivery systems for sustained and localized drug delivery.

Transdermal drug administration generally refers to topical applications of agents to healthy intact skin either for localized treatment of tissues underline the skin of for systemic therapy. For transdermal product, the goal of dosage design

is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin.

Transdermal drug delivery has main advantage over the oral route of administration such as improving patient compliance in long term therapy, by-passing first pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimizing the inter and intra-patient variability, and making it possible to interrupt or terminate when necessary.

Transdermal route will eliminate the side effects associated with oral administration of sitagliptin phosphate. The aim of this present study was to formulate and evaluate sustained release microsphere gel loaded sitagliptin phosphate in order to maintain a sustained drug concentration in serum for longer period of time, which may result in enhanced absorption and thereby improved bioavailability.

CHAPTER – V

PLAN OF WORK

PLAN OF WORK

1. STANDARD CURVE FO SITAGLIPTIN PHOSPHATE

- Preparation of calibration curve
- Estimation of absorption maximum
- Preparation of standard curve

2. PREFORMULATION STUDIES

- Fourier transform infra-red spectroscopic
- Differential Scanning Calorimetric studies

3. FORMULATION OF SITAGLIPTIN PHOSPHATE MICROSPHERES

The microspheres are prepared by non-aqueous solvent evaporation method.

4. CHARACTERIZATION OF SITAGLIPTIN PHOSPHATE MICROSPHERES

- Determination of drug content
- Determination of mean particle size
- Scanning Electron Microscopic
- Determination of percentage yield
- Determination of encapsulation efficiency
- In-vitro dissolution studies

5. SELECTION OF BEST FORMULATION

6. PREPARATION OF GEL LOADED SITAGLIPTIN MICROSPHERES

7. CHARACTERIZATIION OF GEL LOADED SITAGLIPTIN MICROSPHERES

- Deternmination of pH
- Determination of gel strength
- Determination of spreadability
- Determination of drug content

8. COMPARATIVE IN-VITRO STUDIES

9. STABILITY STUDIES

CHAPTER – VI

MATERIALS AND EQUIPMENTS

MATERIALS AND EQUIPMENTS

LIST OF MATERIALS

Table- 2. List of Materials Used

Materials	Manufacturer
Sitagliptin phosphate	Pure chem Pvt. Ltd. Gujarat
Eudragit RS 100	Universal Scientific Appliances, Madurai.
HPMC E15	Universal Scientific Appliances, Madurai.
Chitosan	Universal Scientific Appliances, Madurai.
Tween 80	Universal Scientific Appliances, Madurai.
Carbopol 934	Universal Scientific Appliances, Madurai.
Triethanolamine	Universal Scientific Appliances, Madurai.
Acetone	Universal Scientific Appliances, Madurai.
Liquid paraffin	Universal Scientific Appliances, Madurai.
Petroleum ether	Universal Scientific Appliances, Madurai.

EQUIPMENTS USED

TABLE-3

EQUIPMENTS NAME	**MANUFACTURERS**
Electronic weighing balance	A & D company, Japan.
UV – Visbile spectrophotometer	Shimadzu corporation, Japan
Infrared Spectroscopy	Spectrum RX-1, Perkin Elmer, German
Differential Scanning Calorimetry	DSC Q 200, USA
Scanning Electron Microscopy	Hitachi X 650, Tokyo,Japan
Optical microscope	Towa Optics, India
Mechanical stirrer	Remi, India
Magnetic stirrer	Remi, India
Environmental stability testing chamber	In Labs equipment(Madras) Pvt.Ltd.

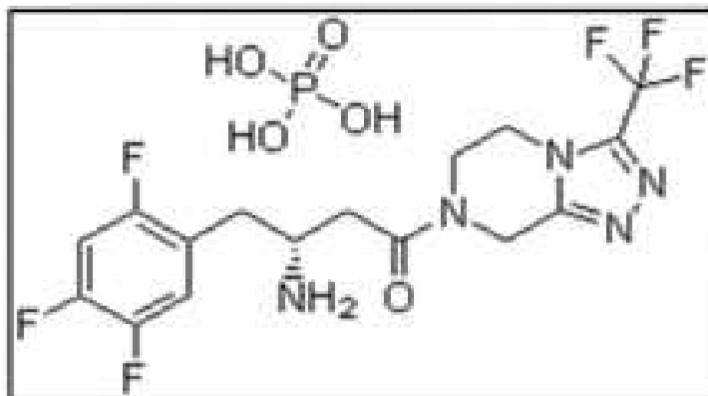
CHAPTER – VII

DRUG PROFILE

DRUG PROFILE

SITAGLIPTIN PHOSPHATE:

Structure:



Chemical Name:

4-Oxo-4- (3-(trifluoromethyl) 5,6 dihydro (1,2,4) triazolo [4,3a] pyrazin-7 (8H) -yl) -1- (2,4,5-trifluorophenyl) butan-2 amine phosphate

Empirical Formula :

C16H15F6N5O.H3PO4

Molecular Weight :

505.31gm/mol

Description :

white to off-white crystalline powder

Melting Point:

198 – 202°C

Category:

Hypoglycemic agent DPP-4 inhibitor.

Brands:

Januvia, Janumet

Dose :

25 to 100 mg daily, in divided doses or once daily.

PKa:

12.4

pH:

pH of 1% aqueous solution of drugs is 6.68

Solubility:

Freely soluble in water, slightly soluble in ethanol (95%) practically insoluble in acetone, chloroform, dichloromethane and ether.

BCS Classification:

It is a BCS class III (high solubility, low permeability)**/**borderline class I (high solubility, high permeability) drug

Sitagliptin Phosphate Pharmacokinetics

Absorption

Absolute bioavailability 87%.

Rapidly absorbed following oral administration; at steady state (within 3 days of therapy initiation), peak plasma concentrations generally attained ≤3 hours following administration of recommended doses.

Onset

Reduction in postprandial plasma glucose excursion: Approximately 60 minutes.

Duration

Approximately 80% inhibition of DPP-4 activity persists for 12 or 24 hours following administration of ≥50 or ≥100 mg, respectively, of sitagliptin.

Food

Food does not appear to affect absorption.[1]

Special Populations

Renal impairment results in increased plasma AUC.Removed modestly by hemodialysis; time to peak plasma drug concentration increased in a limited number of patients with end-stage renal disease requiring hemodialysis.

Moderate hepatic impairment results in increased peak plasma concentrations and AUC; not considered clinically important.

In geriatric patients, modest increases in plasma concentrations compared with younger adults.

Distribution

Distributed into milk in rats; not known whether distributed into human milk.

Plasma Protein Binding

38%.

Metabolism

Metabolized to a limited extent by CYP isoenzymes 3A4 and 2C8 to inactive metabolites.

Elimination Route

Eliminated principally by kidneys via active tubular secretion. Excreted in urine (87%) mainly as unchanged drug and in feces (13%).

Half-life

12.4 hours.

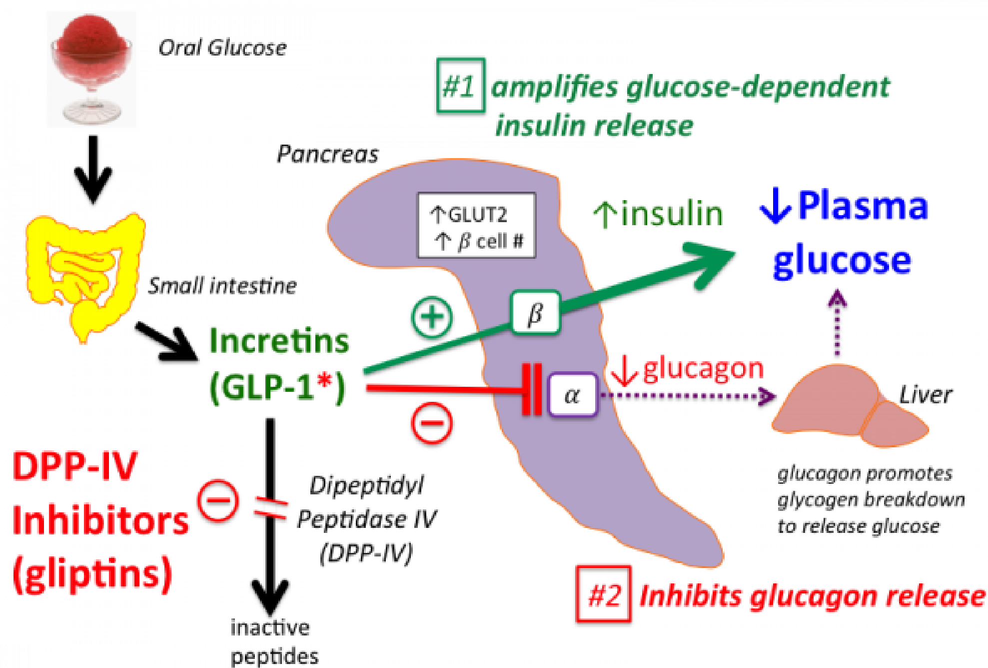
Special Populations

Renal impairment results in increased terminal elimination half-life.

Storage

20–25°C (may be exposed to 15–30°C).

Actions



- Inhibits DPP-4, an enzyme that inactivates incretin hormones glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).
- Inhibits DPP-4 selectively with no effect on DPP-8 or DDP-9 in vitro at concentrations approximating those from therapeutic dosage.
- Increases circulating concentrations of GIP and GLP-1 in a glucose-dependent manner. Coadministration of sitagliptin and metformin has an additive effect on active GLP-1 concentrations.
- GIP and GLP-1 stimulate insulin synthesis and release from pancreatic β-cells in a glucose-dependent manner (i.e., when glucose concentrations are normal or elevated) by intracellular signaling pathways involving cyclic 3′,5′-adenosine monophosphate (cAMP).
- GLP-1 also decreases glucagon secretion from pancreatic α-cells in a glucose-dependent manner, leading to reduced hepatic glucose production.
- Lowers fasting plasma glucose concentrations and reduces glucose excursions following glucose load or meal in patients with type 2 diabetes mellitus.
- Sitagliptin usually not associated with hypoglycemia or substantial changes in body weight.

Cautions for Sitagliptin Phosphate

Contraindications

- Known serious hypersensitivity (e.g., anaphylaxis, angioedema) to sitagliptin or any ingredient in formulation.

Warnings/Precautions:

- Pancreatitis and Pancreatic Precancerous Changes
- Worsening of Renal Function
- Severe Arthralgia

- Sensitivity Reactions
- Loss of Glycemic Control
- Macrovascular Outcomes

Pregnancy

Category B.

Lactation

Distributed into milk in rats; not known whether distributed into human milk.

Pediatric Use

Safety and efficacy of sitagliptin alone or in fixed combination with metformin not established in children <18 years of age.

Geriatric Use

No substantial differences in safety and efficacy relative to younger adults, but increased sensitivity cannot be ruled out.

Substantially eliminated by kidneys; assess renal function prior to initiation of therapy and periodically thereafter because geriatric patients more likely to have decreased renal function.

Renal Impairment

Substantially eliminated by kidneys; assess renal function prior to initiation of therapy and periodically thereafter.

Common Adverse Effects

Sitagliptin monotherapy or add-on therapy with metformin and/or a thiazolidinedione or glimepiride: Nasopharyngitis, upper respiratory tract infection, peripheral edema, headache.

Interactions for Sitagliptin Phosphate

Metabolized to a limited extent by CYP isoenzymes 3A4 and 2C8 to inactive metabolites.

Drugs Metabolized by Hepatic Microsomal Enzymes

Does not inhibit CYP isoenzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4 in vitro or induce CYP3A4. Pharmacokinetic interactions with drugs metabolized by these isoenzymes unlikely.

Inhibitors of P-glycoprotein Transport System

Substrate of p-glycoprotein transport system. Potential pharmacokinetic interaction (increased absorption and renal clearance of sitagliptin) with p-glycoprotein inhibitors.

Clinically important pharmacokinetic interactions with p-glycoprotein inhibitors unlikely. Does not appear to inhibit p-glycoprotein transport system.

Drugs Secreted by Renal Tubular Cationic Transport

Substrate of organic anion transport system; pharmacokinetic interaction unlikely with substrates of organic cationic transport system.

Protein-bound Drugs

Pharmacokinetic interaction unlikely.

There are several therapeutic options for the treatment of type 2 diabetes, which can include lifestyle modification with diet and exercise, as well as drug therapy. They are,

TABLE-4

Insulin Sensitizers	***Biguanides*** (Metformin, Buformin, Phenformin) ***TZDs/"Glitazones" (PPAR)*** (Pioglitazone, Rivoglitazone, Rosiglitazone, Troglitazone) ***Dual PPAR agonist (***Aleglitazar, Muraglitazar, Tesaglitazar)
Insulin Secretagogues	***K+ ATP, Sulfonylureas*** (1st generation: Acetohexamide, Carbutamide, Chlorpropamide, Metahexamide, Tolbutamide, Tolazamide) ***K+ ATP, Sulfonylureas*** (2nd generation: Glibenclamide(Glyburide), Glibornuride, Glipizide, Gliquidone, Glisoxepide, Glyclopyramide, Glimepiride, Gliclazide) ***K+ ATP, Meglitinides/"glinides"*** (Nateglinide, Repaglinide, Mitiglinide) GLP-1 agonists (Exenatide, Liraglutide, Taspoglutide, Albiglutide, Lixisenatide) ***DPP-4 inhibitors*** (Alogliptin, Gemigliptin, Linagliptin, Saxagliptin, Sitagliptin, Vildagliptin)
Analogs/Other Insulins	***Fast-acting*** (Insulin Lispro, Insulin aspart, Insulin Glulisine) ***Short-acting*** (Regular Insulin) ***Long-acting*** (Insulin Glargine, Insulin Detemir, NPH Insulin) ***Ultra-long-acting*** (Insulin Degludec) ***Inhalable*** (Exubera)
AlphaGlucosidase Inhibitors	Acarbose, Miglitol, Voglibose
Amylin Analog	Pramlintide
SGLT2 Inhibitors	Canagliflozin, Dapagliflozin, Empagliflozin, Remogliflozin, Sergliflozin, Tofogliflozin
Other	Benfluorex, Tolrestat

CHAPTER – VIII

EXCIPIENTS PROFILE

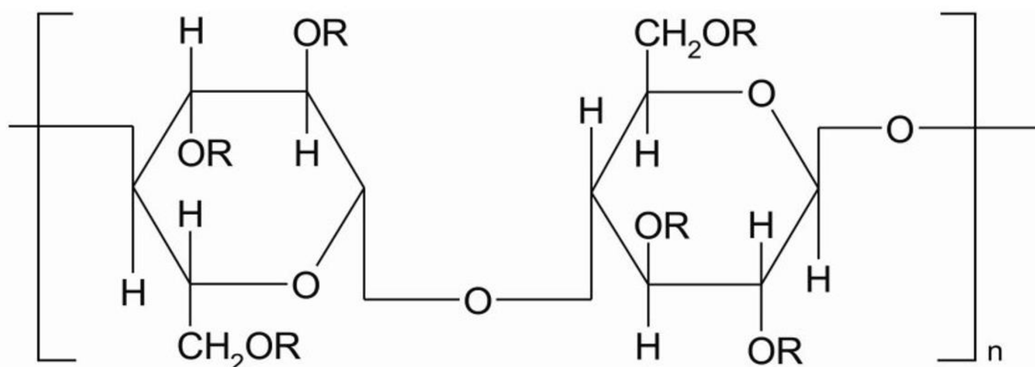
EXCIPIENTS PROFILE

HYDROXYPROPYL METHYLCELLULOSE

Description : Odourless, tasteless, white or creamy-white coloured fibrous or granular powder.

Molecular weight : Approximately 10,000 - 1,500,000.

Structural formula :



Applications : Primarily in the oral products hydroxypropyl methylcellulose was used as a tablet binder, extended release tablet matrix, suspending and thickening agent particularly in ophthalmic preparations. It is widely used in cosmetics and food products and also used as a wetting agent for hard contact lenses and as an adhesive in plastic bandages.

Acidity / Alkalinity : p^H = 5.5 - 8.0 for a 1% w/w aqueous solution.

Density (tapped) : 0.50 - 0.70 g / cm^3 for pharmacoat.

Melting point : Browns at 190-200°C. Glass Transition Temperature is 170 - 180°C.

Solubility : Soluble in cold water and practically insoluble in ethanol (95%), ether and chloroform. But soluble in mixtures of methanol and dichloromethane and mixtures of ethanol and dichloromethane.

Stability : Its powder is a stable material although it is hygroscopic after drying.

Incompatibilities : Incompatible with some oxidizing agents and will not complex with metallic salts and ionic organics.

Safety : Extensively used in cosmetics, food products and widely used as an excipient in oral and topical formulations.

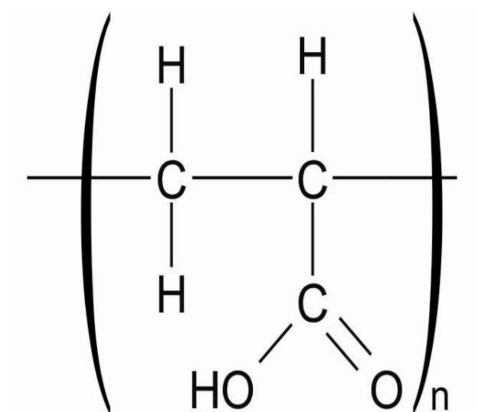
CARBOPOL

Chemical name : Carboxy polymethelene

Empirical formula : (C3H4O2) x (-C3H5-sucrose) y

Grades : 907, 910, 934, 934P, 940, 941, 971P, 974P, 980.

Structural formula :



Description : White, fluffy, acidic, hygroscopic powder with a slight characteristic odour.

Molecular weight : 1 x 106 to 4 x 106.

Density : Bulk: 5 g / cm^3.

Tapped: 1.4 g / $cm^{3.}$

Viscosity : 29,400 to 39,400 cps at 2 ^oC (0.5% neutralized aqueous solution).

pH : 2.5 - 3.0 (1% aqueous solution)

Solubility : Soluble in water, alcohol and glycerin.

Stability : It is relatively unaffected by temperature variations, not subjected to hydrolysis or oxidation and is resistant to bacterial growth.

Applications : It is excellent thickening, emulsifying, suspending and gelling agent. It is used as a tablet binder in sustained release formulations affording zero- to near zero order release. It is used as the bioadhesive component in mucoadhesive ointments, gels and tablets.

Safety : No primary irritation or any evidence of allergic reactions has been observed in human beings following topical application.

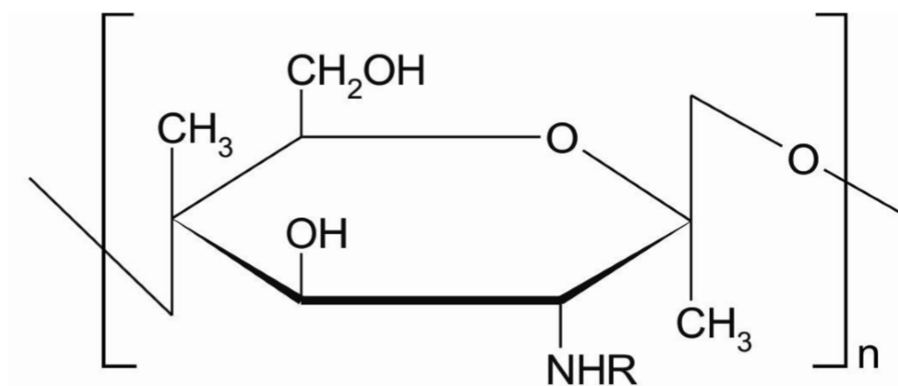
Incompatibility :It is observed with cationic polymers, phenols, resorcinol and high concentration of electrolytes.

CHITOSAN

Chemical name **:** Poly-b-(1,4)-2-Amino-2-deoxy-D-glucose

Description **:** Odourless, white or creamy-white powder or flakes.

Structural formula **:**



Molecular formula **:** C6H11NO4n

Molecular weight **:** 10,000 - 1,000,000

Solubility **:** Easily soluble in water.

Moisture content **:** 10%

Ash content **:** 2.0%

Applications **:** Excellent film forming agent, additives, cosmetics and good bacterial inhibitor. Also used for the preservation of fruits and vegetables. It is widely applied for improving immunity, activating cells, preventing cancer, decreasing blood fat and blood

pressure, adjusting blood sugar, fighting against ageing and adjusting organism environment.

Incompatibilities **:** Incompatible with strong oxidizing agents.

Stability **:** Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying.

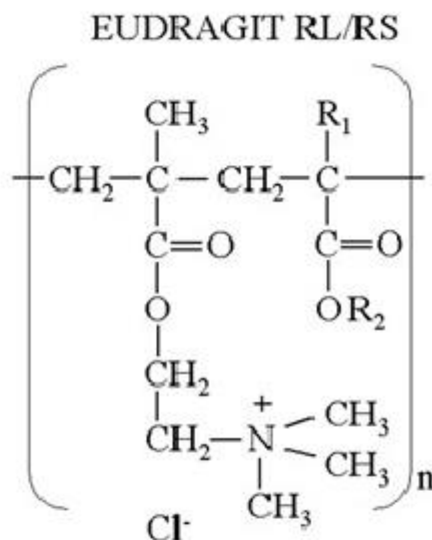
Safety **:** Widely for use as an excipient in oral, cosmetics and other pharmaceutical formulations. Generally regarded as a nontoxic and nonirritant material. It is biocompatible with both healthy and infected skin and shown to be biodegradable.

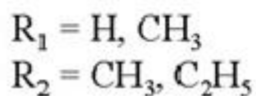
EUDRAGIT- RS100

Molecular weight : Is approximately 135,000.

Description : White powders with a faint characteristic odour.

Structural formula :





Solubility : Dissolves in methanol, ethanol, aqueous isopropyl alcohol and acetone. Practically insoluble in ethyl acetate, methylene chloride, petroleum ether and water

Particle size : At least 95%, less than 0.25 mm.

Film formation : When poured onto a glass plate, a clear film forms upon evaporation of the solvent.

Applications : Polymethacrylates are primarily used in oral capsule and tablet formulations as film coating agents. Depending on the type of polymer used, films of different solubility characteristics can be produced. Eudragit acrylic resins exhibit a broad spectrum of physicochemical properties and are used in a variety of pharmaceutical applications, such as film coating of oral formulations and preparation of controlled release drug systems.

Stability : Minimum stability dates are given on the product labels and batch-related certificates of analysis.

Storage : Protect from warm temperatures and against moisture.

Safety : Polymethacrylate co-polymers are widely used as film coating materials in oral pharmaceutical formulations. They are also used to a lesser extent in topical formulations and are generally regard as non toxic and non-irritant materials.

CHAPTER – IX

EXPERIMENTAL PROTOCOL

EXPERIMENTAL PROTOCOL

Preformulation study:

Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of pre-formulation testing is to generate information useful to the formulator in developing stable and bio available dosage forms that can be man produced.

The following preformulation studies are carried out:

- Description
- Finding the absorption maxima
- Standard curve
- Infra-red spectroscopy studies (compatibility studies)
- Differential Scanning Calorimetry

Description

About 1g of sample is taken in a dry petridish and the sample is observed for compliance against the specification.

Identification of the drug: Finding the absorption maxima (λ max)

The absorption maxima were found for drug identification. Ultraviolet Visible spectrophotometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the visible/Ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption.

The various batches of the microspheres were subjected for drug content analysis. Accurately weighed microsphere samples were mechanically powdered.

The powdered microspheres were dissolved in adequate quantity of phosphate buffer PH 7.2 then filter. The UV absorbance of the filtrate was measured using a UV spectrometer at 267nm.

Fourier transform infrared (FTIR) spectral analysis

FT-IR is used to identify the functional groups in the molecule. The drug is mixed with KBr and pellet is formed. Each KBr disk was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 400 to 4,500 cm−1. The characteristic peaks were recorded. The results are shown in the following figure. and table.

Drug-Excipient Compatibility studies by FT-IR

Fourier infrared spectroscopy (FT-IR) analysis was performed for the pure drug and physical admixtures (Polymers, excipients) individually and then the drug and physical admixtures are mixed together and FT-IR is taken to find out that there is no interaction between drug, polymers and the excipients. The results are shown in the following figure.and table.

Differential Scanning Calorimetry(DSC):

Differential scanning calorimetry or DSC is a thermo analytical technique in which difference in the amount of heat required to increase the temperature of a sample and reference as a function of temperature. Both the sample and reference mentioned at nearly sample temperature throughout the experiment.

Mehtod:

Accurately weighed sitagliptin phosphate and formulations were analyzed using an automatic thermal analyzer by using an automatic thermal analyzer system (DSC60 Shimadzu Corporation Japan). Sealed and perforated aluminium pans were used in the experiments for all the samples. Temperature calibrations were performed using indium as a reference. The entire sample were run at a scanning rate of 10°c/min from $50\,^{o}$c-$300\,^{o}$c.

Standard Curves For Sitagliptin Phosphate :

Preparation of O.1N HCL:(Indian Pharmacopoeia 2010)

8.5 ml of Hydrochloric acid was dissolved in 1000ml of distilled water.

Preparation of Phosphate Buffer pH 7.2:

A known volume of 0.2M potassium dihydorgen phosphate is placed in a 200ml volumetric flask. 22.4ml of 0.2M sodium hydroxide is added and make up to the volume with distilled water.

0.2M Pottasium Dihydrogen Phosphate:

A known quantity(27.218g) of potassium dihydorgen phosphate is dissolved and diluted to1000ml with water

0.2M Sodium Hydroxide:

A known quantity (8g) of sodium hydroxide is dissolved and make up to 1000 ml with water.

Estimation of absorption maximum: (λ max)

The sitagliptin phosphate drug solutions (5,10,15,20 and 25µg/ml) were taken in a standard cuvette and scanned in the range of 200-400nm by using UV-Spectrophotometer. The absorbance of each sample was measured at 267nm. The standard curve is plotted against absorbance and concentration of the sample. Similarly, the procedure can be repeated for phosphate buffer pH 7.2. and for 0.1N HCL..

FORMULATION OF SITAGLIPTIN PHOSPHATE MICROSPHERES:

The microspheres containing the anti-diabetic drug, sitagliptin phosphate, as the core material were prepared by a **non-aqueous solvent evaporation method. (Garud and Garud.,2012)**

The drug and the plymers were mixed with acetone at various ratos. The slurry was introduced into 100ml of liquid paraffin while stirring with a mechanical stirrer equipped with a three-blade propeller at room temperature. The solution was stirred for 4 hours to allow the solvent to evaporate and the microspheres were collected by filtration by Whatman filter paper no. 1. The microspheres were washed repeatedly with petroleum ether(40°c-60°c) until free from oil. The microspheres were collected and dried for 3hours at room temperature stored in a dessicator over fused calcium chloride.

CHARACTERIZATION OF SITAGLIPTIN MICROSPHERES:

Determination of mean particle size:

The particle size was measured using an optical microscope, and the mean particle size was calculated by measuring 300 particles with the help of a calibrated ocular micrometer. A small amount of dry microspheres was suspended in liquid paraffin (10 ml). A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and diameter of at least 300 particles was measured using a calibrated optical micrometer.

Scanning Electron Microscopy:

The purpose of the Scanning Electron Microscopy study was to obtain a topographical characterization of micropsheres. The micropsheres were mounted on brass stubs using double-sided adhesive tape. Scanning electron microscopy photographs were taken with a scanning electron microscope (JSM-5610LV, Joel Ltd, Tokyo, Japan) at the required magnification at room temperature. The working distance of 39 mm was maintained, and the acceleration voltage used was 15 kV, with the secondary electron image as a detector.

Yield of Microspheres

The prepared Microspheres were collected and weighed. The actual weight of obtained Microspheres divided by the total amount of all non-volatile material

that was used for the preparation of the Microspheres multiplied by 100 gives the % yield of Microspheres.

This was calculated by the use of following formula.

% yield= (Actual weight of the product / Total weight of excipients and drug) × 100

Determination of Drug Content and Encapsulation Efficiency

100 mg of accurately weight microspheres were suspended in a phosphate buffer pH 7.2 upto 24 hours. Next day, the sample was shaken using mechanical shaker for few hours. Then it was filtered and from the filtrate, few ml of aliquot was taken and made the suitable dilutions and analyzed for the drug content at 267 nm by spectrophotometry. The % of Encapsulation Efficiency was calculated. Taking into the consideration the dilution factor and the amount of micro beads takes, the amount of drug encapsulation per unit weight of micro beads were calculated. The drug encapsulation efficiency was calculated using for the formula

Encapsulation efficiency=practical drug content/theoretical drug content*100

Evaluation of in vitro release by static method:

Microspheres, equivalent to 25 mg of sitagliptin phosphate, were accurately weighed and transferred to beaker containing 100 ml phosphate buffer (pH 7.2). The flask was kept in a magnetic stirrer at 37°c, 1 ml samples withdrawn at regular intervals and, after suitable dilution, the amount of drug released was determined using a spectrophotometer at 267 nm. Following each sample withdrawal, 1 ml of phosphate buffer was added to the release medium to replenish it. The microspheres were allowed to settle down and clear supernatant medium withdrawn for drug analysis. Samples were collected for every 1 hour and the study was carried over for 12 hours. The sample was filtered and the microspheres collected were transferred to the dissolution flask. (**Karthikeyan K., et al., 2012)**

Kinetics of drug Release:

Several theories and kinetic models describe the dissolution of drug from immediate release and modified release dosage forms. There are several models to represent the drug dissolution profiles where f (t) is a function of time related to the amount of drug dissolved from the pharmaceutical dosage form.

The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the usage of a generic equation that mathematically translates the dissolution curve function of some parameters related with the pharmaceutical dosage forms. Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time'‘t’ or Q(t). Some analytical definitions of the Q(t) function are commonly used, such as zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell models, Weibull models. These models are used to characterize drug dissolution/release profiles.

(i) Zero Order Kinetics

This model represents an ideal release profile in order to achieve the pharmacological prolonged action. Zero order release constitutes drug release from the dosage form that is independent of the amount of drug in the delivery system (that is, a constant release rate). The following equation is used to express the model:

$$Qt = Qo + Kot$$

Where,

Qt is the amount of drug dissolved in time t

Qo is the initial amount of drug in the solution

Ko is the zero order release constant

For practical purposes the equation is rearranged:

Percent drug released = Kt

This is applicable to dosage forms like transdermal systems, coated dosage forms, osmotic systems as well as matrix tablets with low soluble drugs.

(ii) First Order Kinetics

First order release constitutes drug release in a way that is proportional to the amount of drug remaining in its interior; in such a way that amount of drug released by unit time diminish. The following equation is used to express the model:

log Qt = log Qo + Kt/2.303

Where,

Qt is the amount of drug dissolved in time t

Qo is the initial amount of drug in the solution

K is the first order release constant

For practical purposes the equation is rearranged:

Log % of drug unreleased = Kt/2.303

This model is applicable to dosage forms such as those containing water soluble drugs in porous matrices.

(iii) Higuchi Model

Higuchi describes drug release as a diffusion process based in Fick's law,square root dependent. The following equation is used to express the model:

Qt = Kht1/2

Where,

Qt is the amount of drug dissolved in time t

Kh is the first order release constant

For practical purposes the equation is rearranged:

Percent drug released = $Kt^{1/2}$

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

(iv) Peppas-Korsmeyer Model:

This model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved

The following equation is used to express the model

$$Q_t/Q_\infty = Kt^n$$

Where,

Q_t is the amount of drug dissolved in time t

Q_∞ is the amount of drug dissolved in infinite time

n is the release exponent indicative of drug release mechanism K is the kinetic constant

For practical purposes the equation is rearranged:

Log percent drug released = log k +n log t

Peppas used n value in order to characterize different release mechanism concluding for values of n = 0.5 for Fickian diffusion and values of n, between 0.5 to 1.0 for anomalous transport (corresponds to diffusion, erosion and swelling mechanism or mixed order kinetics) and higher values of n, n=1 or n>1 for case-II transport (corresponds to erosion and relaxation of swollen polymer layer).

PREPARATION OF GEL:

Accurately weighed sitagliptin phosphate miccrospheres(drug equivalent) was added to 15ml of water in a beaker and stirred well, 400 mg of carbopol was added to that microspheres dispersion, while stirring at 250 rpm, to obtain a homogenous mixture. Stirring was continued until a lump free suspension was obtained. Thereafter, 0.33ml of triethanolamine was added to produce a gel. (**Karthikeyan K., et al., 2012)**

Evaluation of microspheres-loaded gel:

Determination of pH:

Determination of pH was done by Indosati Digital pH meter pH meter was calibrated before use using standard buffer solution.

Gel strength determination:

A composition of 50 g of microspheres-loaded gel was taken in a 10 ml beaker. The weight of 1000 mg for measuring gel strength was allowed to penetrate into the gel. The gel strength, which means the viscosity of the gel, was determined by time (seconds)

Spreadability

A sample of 0.5 g of each formulation was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations.

Drug content:

About 0.5 g of gel was dissolved in 25 mL phosphate buffer at pH 7,2. It was then filtered after constant stirring and analyzed by UV spectrophotometer after suitable dilution at 267 nm.

Evaluation of in vitro release by dynamic method:

Evaluation of in vitro release studies of sitagliptin phosphate carbomer gel was carried out at 37ºc using phosphate buffer (pH 7.2) as the release medium. A glass tube of 10 mm diameter and 100 mm height was taken. One end of the tube was closed using an dialysis membrane with the help of adhesive tape while the other end was kept open and used as drug reservoir compartment. Gel (1 g) containing sitagliptin phosphate was accurately weighed and transferred to the glass tube in a vertical position through the open end. The gel was gently pushed down to the surface of the dialysis membrane with the help of a stainless steel spatula to ensure that all the gel was in contact with the membrane. Phosphate buffer (2 ml, pH 7.2) was added to the reservoir compartment to wet the gel. The glass tube was placed in a beaker containing 100 ml of phosphate buffer (pH 7.2) such that that the dialysis membrane is just immersed in the phosphate buffer which acted as the receiving compartment. The receiving compartment was magnetically stirred (100 rpm, Remi, India) at 37ºc. Samples (1 ml) were withdrawn from the receiving compartment at regular 1hour intervals and the amount of sitagliptin phosphate released from the gel was determined using a spectrophotometer at 267 nm. After each withdrawal of sample, an equal quantity of phosphate buffer was added to the receiving compartment to replenish it. Samples were collected for every 1 hour and the study was carried over for 12 hours. (**Karthikeyan K., et al., 2012)**

Stability Study

Accelerated Stability Studies

Stability

Stability is officially defined as the time lapse during which the drug product retains the same property and characteristics that it possessed at the time of manufacture. This process begins at early development phases.

Definition Stability of a pharmaceutical preparation can be defined as “the capability of a particular formulation (dosage form or drug product) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life.

Instability in modern formulation is often undetectable only after considerable storage period under normal conditions. To assess the stability of a formulated product it is usual to expose it to high stress conditions to enhance deterioration and therefore the time required for testing is reduced. Common high stress factors are temperature and humidity. This will eliminate unsatisfactory formulation.

Purpose of stability testing:

- To study of drug decomposition kinetics
- To develop stable dosage form
- To establish the shelflife or expiration date for commercially available drug product.
- To ensure the efficacy, safety and quality of active drug substance and dosage forms.

Table - 5 Stability Conditions Chart

S.NO	STUDY	STORAGE CONDITION	MINIMUM PERIOD
1	Long term	25ºC ± 2ºC 60% ± 5% RH	12 months
2	Intermediate	30ºC ± 2ºC 65% ± 5% RH	6 months
3	Accelerated	40ºC ± 2ºC 75% ± 5% RH	6 months

The stability studies of formulation F1 to F15 were carried out at 45° C ± 2°C 75% ± 5% RH and leakage of the drug from the microspheres were analyzed in terms of percentage drug content.

CHAPTER – X

RESULTS AND DISCUSSION

TABLES AND FIGURES

RESULTS AND DISCUSSION

PREFROMULATION:

**Description - ** White, Odourless powder.

STANDARD CURVES FOR SITAGLIPTIN PHOSPHATE:

The calibration medium pH (7.4) were prepared by using phosphate buffer and 0.1 NHCL were as per the I.P procedure(I.P 2010)

Estimation of absorption maximum (λmax):

The λmax of sitagliptin phosphate was estimated by scanning the 10µg/ml concentration of the drug solution in buffer solution of phosphate buffer pH (7.4) and λmax of 267nm in phosphate buffer solution and λmax of 267nm in 0.1N HCL. The results were shown in table-5a and table-5b.

Preparation of Standard curves:

The standard curves of sitagliptin phosphate prepared by using phosphate buffer pH (7.4) and 0.1N HCL. The linear correlation coefficient was found to be 0.999605 and sitagliptin obeys the Beer's law within the concentration range of 5-25µg/ml.

IR spectra study:

From IR spectra of sitagliptin, physical mixture of drug and polymer, and Microspheres it can be seen that there is no significant change in IR spectra of microspheres i.e., it is nearly same to that of plain compounds. IR Spectra is shown in figure-5 a, b, c, d.

DSC spectra study:

The DSC thermogram of sitagliptin base exhibited an exothermic peak at about 208.76^{o}C corresponding to its melting point. But in thermal analysis, sitagliptin-loaded microspheres show peak originating from sitagliptin only slighty

difference in peak of temp. From DSC spectra of sitagliptin peak shown, and microspheres shown peak at, near to drug peak indicate that there is no significant change in DSC spectra of microspheres i.e., it is nearly same to that of plain compounds. DSC Spectra is shown in figure 6-a,b.

Formulation of Sitagliptin Phosphate Microspheres:

The sitagliptin microspheres were prepared by non-aqueous solvent evaporation method. The microspheres were prepared by using Eudragit rs 100 in different ratios. After conducting five trial batches(F1-F5) it was optimized by characterization and changing the parameters such as stirring speed, rpm, polymer ratios and (F6-F10) batches were prepared along with HPMC E15 and (F11-F15) were prepared along with chitosan as a rate retardant polymers.

Characterization of Sitagliptin Phosphate Microspheres:

All the formulations were evaluated for its drug content, mean particle size, encapsulation efficiency and drug loading.

Determination of Drug content:

Drug content of all formulation was found in range of 52.08% to 97.92% and its efficiency slightly decreases with increasing the HPMC content. The extent of loading influenced the particle size distribution of microspheres. When the distribution coefficient was high, efficiency of drug entrapment into microspheres was elevated. It is already reported that the size of microspheres depends upon various factors such as viscosity of the dispersed phase and dispersion medium, temperature, speed of stirring, amount and size of porous carrier, etc. So microspheres of desired size can be obtained by varying these factors.

Encapsulation Efficiency:

The encapsulation efficiency of formulation F1 to F15 was carried out and found to be in a range 54.17% to 88.88 %.

Particle characteristics:

SEM morphology:

Scanning electron microphotographs showed that the microspheres were spherical with a smooth to rough surface (Fig 19). Pores were observed on the microsphere surface.

Mean particle size:

Increasing polymer concentrations (i.e., drugpolymer ratio from 1:1 to 1:4) increased microsphere size from 54.51to 92.62 61.42 µm for eudragit rs 100, 61.42 to 75.59µm for chitosan, 60.69 to 71.39µm for HPMC. However, in all cases, the increase was not significant .

Increase in stirring rate from 600 rpm to 1400 rpm also seemed to have decreased microsphere size but the decrease was not significant .

It was already cleared that if the size of microspheres is less than 55µm, release rate of drug will be high with reduced floating ability, the average particle sizes of microspheres were between 54.51 to 92.62 µm. It was observed that the mean particle size of the microspheres was significantly decreased with increase in the concentration of HPMC and reduces in the concentration of Eudragit RS100. It may be attributed to the forming of a thicker Eudragit RS100 layer with the increase of concentration of Eudragit RS100 in the medium

In-vitro drug release of Sitagliptin phosphate microspheres:

The dissolution studies was carried out in phosphate buffer for pH 7.2 for 12 hours. The invitro dissolution studies of all formulations were compared with pure drug. The results of in vitro dissolution studies of all formulations form the sitagliptin microspheres were shown in figure 13 a to 13d.

When compared the in vitro release profile of all formulations are significantly greater than that pure drug sitagliptin phosphate.

Formulations (F1E-F5E) using eudragit rs 100 at different ratios shown the percentage drug release of 99.37% at 7 hours, 95.08%at 8 hours,91.85% at 9 hours, 90.96% at 10 hours , 94.48% at 9 hours respectively.

Formulations (F6EH-F510EH) using eudragit rs 100 along with HPMC E15 at different ratios shown the percentage drug release of 90.46% at 8 hours, 90.39%at 10 hours, , 92.63% at 12 hours , 96.07% at 9 hours, 92.20% at 10hours respectively.

Formulations (F11ECH-F15ECH) using eudragit rs 100 along with chitosan at different ratios shown the percentage drug release of 88.26% at 8 hours, 86.84%at 10 hours, , 87.34% at 12 hours , 88.44% at 9 hours,86.13% at 10hours respectively.

Drug release (for 1:3 drug/polymer ratio) after 12 h was in the rank order:

HPMC > ethyl cellulose > chitosan>eudragit rs 100 (Fig 10), with the formulation prepared with HPMC E15 releasing approximately 92.63% of drug after 12 h.

Increase in stirring speed from 600 rpm to 1400 rpm enhanced drug release rate. At 1600 rpm, complete drug release was observed except for chitosan microspheres. Fig 10 shows that the difference in drug release pattern among the various polymer types was not significant .

Kinetic analysis:

The release data was obtained for zero order, first order, higuchi model, Hixson crowell model, korsmeyer-peppas model. The correlation co-efficient of F1E to F15-ECH formulations for zero order, higuchi, Hixson-crowell and first order equations was shown in table-7. Formulation F8-EH was found high correlation to zero order kinetics(0.899, figure 11a to 11c) rather than Hixson-crowell models.

Higuchi model:

The release kinetics of all the formulations is best fitted the higuchi model. Higuchi model with R^2 values from 0.575 to 0.900. From these higuchi model values, the release kinetics showed purely diffusion controlled. The release kinetics are shown in figure 16a to 16c.

Korsmeyer –peppas model:

The drug release was proportional to square root time, indicating that the drug release from polymeric microspheres was diffusion controlled. The data obtained was also put in korsemeyer-peppas equation to find out release exponent (n values between 0.5 to 1.0 for anomalous transport corresponds to diffusion, erosion and swelling mechanism or mixed order kinetics(n value ranges from 0.557 to 0.739), which describes the drug release mechanism by non fickian diffusion.

SELECTION OF BEST FORMULATION:

From the above results characterization, the best formulation of microspheres was selected showing particle size 62.28µm and in vitro drug release 92.63% in 12 hours.

PREPARATION AND CHARACTERIZATION OF GEL:

The sitagliptin phosphate microspheres were incorporated in a conventional carbomer gel formulation. Carbopol 934 is a commercial grade of carbomer. It is gelled by neutralization with triethanolamine. The formulated carbomer gel was very clear, transparent and elegant. Incorporation of gelatin microspheres resulted in finely dispersed particles in the gel system. A loading dose of the free drug was incorporated in the carbomer gel to produce immediate effect while drug-loaded microspheres are added to provide sustaining dose. They were finally characterized for drug content, pH, gel strength, and spreadability and shown appreciable results.(Table-11)

Comparison of invitro dissolution studies of sitagliptin phosphate microsphere gel with marketed formulation:

In-vitro drug release profile for sitagliptin phosphate of phosphate buffer of pH 7.2 for 12 hours, sitagliptin microsphere gel showed greater sustained action(12hours) when compared with sitagliptin marketed formulation(1hour). The results were shown in table-6d.

Stability studies:

The formulations F15 was selected for stability studies on the basis of their encapsulation efficiency, drug content, and high cumulative %drug release. The stability studies carried out at 25°c (room temperature) and 40 ^{o}c/75%RH for the best formulation up to 30 days. In 15 day time interval, the microspheres were analysed for drug content, encapsulation efficiency and cumulative % drug release. The formulation showed not much variation in any parameter. The results are shown in figure 20a-c.

TABLE- 5a CALIBRATION OF SITAGLIPTIN PHOSPHATE IN 0.1N HCL

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE(nm
1	5	0.011±0.0021
2	10	0.031±0.0016
3	15	0.05±0.0016
4	20	0.07±0.0012
5	25	0.089±0.0002

Regression value = 0.999605

TABLE-5b CALIBRATION OF SITAGLIPTIN PHOSPHATE IN PHOSPHATE BUFFER P^H 7.2

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE(nm
1	5	0.021±0.0024
2	10	0.045±0.0019
3	15	0.069±0.0026
4	20	0.092±0.0031
5	25	0.116±0.0045

Regression value=0.9999975

TABLE -6 FORMULATION OF SITAGLIPTIN PHOSPHATE MICROSPHERES

FORMULATION	BATCH CODE														
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**
SITAGLIPTIN (mg)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
EUDRAGIT RS 100 (mg)	0.25	0.50	0.75	1.00	1.25	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HPMC E-15 (mg)	-	-	-	-	-	0.10	0.20	0.30	0.40	0.50	-	-	-	-	-
CHITOSAN (mg)	-	-	-	-	-	-	-	-	-	-	0.10	0.20	0.30	0.40	0.50
ACETONE (ml)	6.00	9.00	12.0	15.0	18.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
TWEEN 80 (%)	0.10	0.30	0.50	0.70	0.90	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
STIRRING SPEED(RPM)	600	800	1000	1200	1400	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200

TABLE-7 DRUG CONTENT AND MEAN PARTICLE SIZE FOR SITAGLIPTIN PHOSPHATE MICROSPHERES

S.NO	FORMULATION CODE	DRUG CONTENT (%)	MEAN PARTICLE SIZE(µm)
1.	F1-E	60.42	92.62
2.	F2-E	70.83	85.52
3.	F3-E	81.25	76.35
4.	F4-E	83.33	56.35
5.	F5-E	75	54.51
6.	F6-EH	60.42	60.69
7.	F7-EH	66.66	61.63
8.	F8-EH	97.92	62.28
9.	F9-EH	81.25	66.54
10.	F10-EH	77.08	71.39
11.	F11-ECH	52.08	61.42
12.	F12-ECH	60.41	65.39
13.	F13-ECH	62.5	64.16
14.	F14-ECH	66.67	71.94
15.	F15-ECH	75	75.59

TABLE- 8 PERCENTAGE YIELD, DRUG CONTENT, THEORETICAL DRUG LOADING, EXPERIMENTAL DRUG LOADING AND ENCAPSULATION EFFICIENCY OF SITAGLIPTIN PHOSPHATE MICROSPHERES

S.NO	F.CODE	%YIELD (%)	THEORETCIAL DRUG LOADING (%)	EXPERIMENTAL DRUG LOADING (%)	ENCAPSULATION EFFICIENCY (%)
1.	F1-E	39.91	50	70.47	56.25
2.	F2-E	46.74	33.33	49.02	68.75
3.	F3-E	56.79	25	33.93	77.08
4.	F4-E	63.39	20	26.29	83.33
5.	F5-E	69.86	16.67	18.55	77.77
6.	F6-EH	65.73	18.51	16.43	58.33
7.	F7-EH	67.24	17.24	17.98	70.13
8.	F8-EH	70.89	16.12	20.22	88.88
9.	F9-EH	72.79	15.15	16.62	79.86
10.	F10-EH	77.54	14.28	13.94	75.69
11.	F11-ECH	66.71	18.51	15.03	54.17
12.	F12-ECH	69.05	17.24	15.25	61.11
13.	F13-ECH	71.79	16.12	14.51	64.58
14.	F14-ECH	72.37	15.15	14.82	70.83
15.	F15-ECH	74.86	14.29	14.70	77.08

TABLE 9a IN-VITRO DISSOLUTION PROFILE FOR SITAGLIPTIN PHOSPHATE MICROSPHERES

TIME IN HOURS	CUMULATIVE % DRUG RELEASE									
	FORMULATION CODE- EUDRAGIT RS 100									
	F1-E		F2-E		F3-E		F4-E		F5-E	
	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD
1.	38.78	0.24	39.98	0.10	39.20	0.82	40.13	0.86	40.73	0.28
2.	39.95	0.53	41.72	0.174	41.30	0.56	42.22	0.81	42.65	0.12
3.	41.87	0.72	44.07	0.76	42.69	0.10	44.57	0.68	44.89	0.43
4.	45.67	0.28	45.70	0.61	44.60	0.10	45.17	0.53	47.19	0.40
5.	54.54	0.72	48.15	0.61	54.12	0.15	46.66	0.72	51.60	0.81
6.	64.16	0.68	59.90	0.61	60.22	0.16	53.83	0.26	62.10	0.13
7.	99.37	0.24	80.14	0.40	76.87	0.10	65.94	0.24	66.79	0.14
8.			95.08	0.38	80.10	0.61	70.52	0.16	74.42	0.28
9.					91.85	0.53	81.27	0.16	94.48	0.13
10.							90.96	0.61		
11.										
12.										

TABLE-9b IN-VITRO DISSOLUTION PROFILE FOR SITAGLIPTIN PHOSPHATE MICROSPHERES

TIME IN HOURS	CUMULATIVE % DRUG RELEASE									
	FORMULATION CODE- EUDRAGIT RS 100 WITH HPMC E15									
	F6-EH		F7-EH		F8-EH		F9-EH		F10-EH	
	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD
1.	38.31	0.51	37.30	0.27	37.94	0.53	37.82	0.81	39.31	0.60
2.	39.14	0.61	38.18	0.16	38.92	0.54	39.60	0.10	40.34	0.10
3.	39.99	0.61	40.63	0.54	40.56	0.10	41.34	0.16	43.11	0.10
4.	43.18	0.61	42.72	0.53	42.51	0.61	43.01	0.10	44.85	0.61
5.	45.66	0.57	43.86	0.46	43.25	0.61	45.21	0.61	45.81	0.44
6.	57.84	0.12	45.53	0.16	44.39	0.90	61.46	0.80	47.80	0.56
7.	80.88	0.61	49.93	0.30	45.38	0.40	80.45	0.58	54.86	0.22
8.	90.46	0.49	59.69	0.14	50.92	0.44	96.07	0.17	60.58	0.21
9.			79.50	0.53	61.93	0.24			78.72	0.68
10.			90.39	0.10	70.37	0.28			92.20	0.21
11.					87.06	0.21				
12.					92.63	0.59				

TABLE-9c IN-VITRO DISSOLUTION PROFILE FOR SITAGLIPTIN PHOSPHATE MICROSPHERES

TIME IN HOURS	CUMULATIVE % DRUG RELEASE									
	FORMULATION CODE- EUDRAGIT RS 100 WITH CHITOSAN									
	F11-ECH		F12-ECH		F13-ECH		F14-ECH		F15-ECH	
	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD
1.	37.93	0.81	37.75	0.74	37.47	0.26	38.39	0.31	38.53	0.37
2.	39.14	0.81	38.96	0.76	38.50	0.16	39.07	0.28	39.78	0.53
3.	40.06	0.86	40.45	0.10	40.59	0.48	40.77	0.36	42.19	0.86
4.	41.98	0.72	42.05	0.10	41.66	0.53	44.14	0.12	44.18	0.10
5.	44.50	0.76	43.57	0.61	42.37	0.63	47.51	0.96	44.92	0.10
6.	50.39	0.52	44.39	0.90	43.43	0.10	52.02	0.25	50.67	0.46
7.	63.49	0.26	45.53	0.54	45.63	0.61	71.05	0.98	56.39	0.58
8.	88.26	0.56	51.35	0.32	48.08	0.61	88.44	0.48	63.77	0.55
9.			60.26	0.21	60.65	0.53			69.84	0.10
10.			71.12	0.21	71.08	0.40			86.13	0.31
11.			86.84	0.69	76.73	0.25				
12.					87.34	0.37				

TABLE-9d IN-VITRO DRUG RELEASE PROFILE FOR SITAGLIPTIN PHOSPHATE

TIME IN HOURS	CUMULATIVE % DRUG RELEASE									
	COMPARISON FOR BEST RELEASE PROFILE									
	GEL		PURE		TABLET		F8-EH		F13-ECH	
	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD
1.	37.68	1.34	95.73	0.46	96.12	1.07	37.94	0.53	37.47	0.26
2.	38.78	0.43					38.92	0.54	38.50	0.16
3.	39.85	0.31					40.56	0.10	40.59	0.48
4.	40.34	0.21					42.51	0.61	41.66	0.53
5.	41.69	0.34					43.25	0.61	42.37	0.53
6.	43.82	0.27					44.39	0.91	43.43	0.10
7.	45.96	0.48					45.38	0.40	45.63	0.61
8.	48.77	0.32					50.92	0.44	48.08	0.61
9.	58.77	0.28					61.93	0.24	60.65	0.53
10.	66.11	0.60					70.37	0.28	71.08	0.40
11.	81.02	0.60					87.06	0.21	76.73	0.25
12.	94.80	0.60					92.63	0.59	87.34	0.37

TABLE-10 IN-VITRO RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES

FORMULATION CODE	ZERO ORDER KINETICS		FIRST ORDER KINETICS		HIGUCHI MODEL		KORSMEYER PEPPAS MODEL		HIXON CROWEL MODEL	
	R^2	$K_O(h^{-1})$	R^2	$K_O(h^{-1})$	R^2	$K_H(h^{-1/2})$	R^2	n^2	R^2	$K_{HC}(h^{-1/3})$
F1-E	0.775	9.877	0.776	-9.877	0.575	-0.366	0.557	0.362	0.575	-0.366
F2-E	0.847	9.020	0.847	-9.020	0.782	-0.273	0.657	0.371	0.782	-0.273
F3-E	0.897	8.199	0.897	-8.199	0.897	-0.236	0.779	0.394	0.897	-0.236
F4-E	0.868	6.869	0.868	-6.869	0.871	-0.195	0.739	0.345	0.870	-0.196
F5-E	0.860	7.654	0.886	-7.489	0.856	-0.233	0.639	0.386	0.856	-0.233
F6-EH	0.852	8.878	0.852	-8.877	0.814	-0.249	0.589	0.306	0.814	-0.249
F7-EH	0.785	5.542	0.786	-5.542	0.900	-0.153	0.639	0.331	0.731	-0.153
F8-EH	0.899	5.551	0.830	-5.550	0.778	-0.256	0.637	0.331	0.777	-0.167
F9-EH	0.835	7.848	0.835	-7.847	0.740	-0.240	0.637	0.369	0.740	-0.241
F10-EH	0.816	6.416	0.816	-6.416	0.767	-0.182	0.654	0.318	0.766	-0.182
F11-ECH	0.793	7.612	0.792	-7.611	0.734	-0.200	0.587	0.319	0.734	-0.200
F12-ECH	0.786	5.142	0.785	-5.141	0.756	-0.134	0.619	0.283	0.756	-0.134
F13-ECH	0.823	5.093	0.825	-5.093	0.813	-0.136	0.645	0.311	0.813	-0.136
F14-ECH	0.791	6.722	0.790	-6.722	0.748	-0.179	0.605	0.308	0.748	-0.179
F15-ECH	0.837	6.023	0.837	-6.025	0.836	-0.156	0.732	0.312	0.836	-0.157

TABLE -11 CHARACTERIZATION OF GEL OF SITAGLIPTIN MICROSPHERES

pH	SPREADABILITY	GEL STRENGTH	DRUG CONTENT
5.01	4.8(g.cm/second)	52 seconds	98.02%

TABLE-12 STABILITY STUDIES

S.NO	FORMULATION CODE	EVALUATION PARAMETERS	BEFORE STORAGE	Stored at 40°C ± 2°C and 75% ± 5% RH (AFTER STORAGE)	
				For 15 days	For 30 days
1.	**F8-EH**	Drug content (%)	97.9	96.8	95.1
2.	**F8-EH**	Encapsulation efficiency(%)	88.88	86.9	85.8
3.	**F8-EH**	% cumulative drug release(%)	92.63	91.5	90.2

FIGURE-4a DETERMINATION OF λmax FOR SITAGLIPTIN PHOSPHATE USING PHOSPHATE BUFFER P^H 7.2 at 267nm

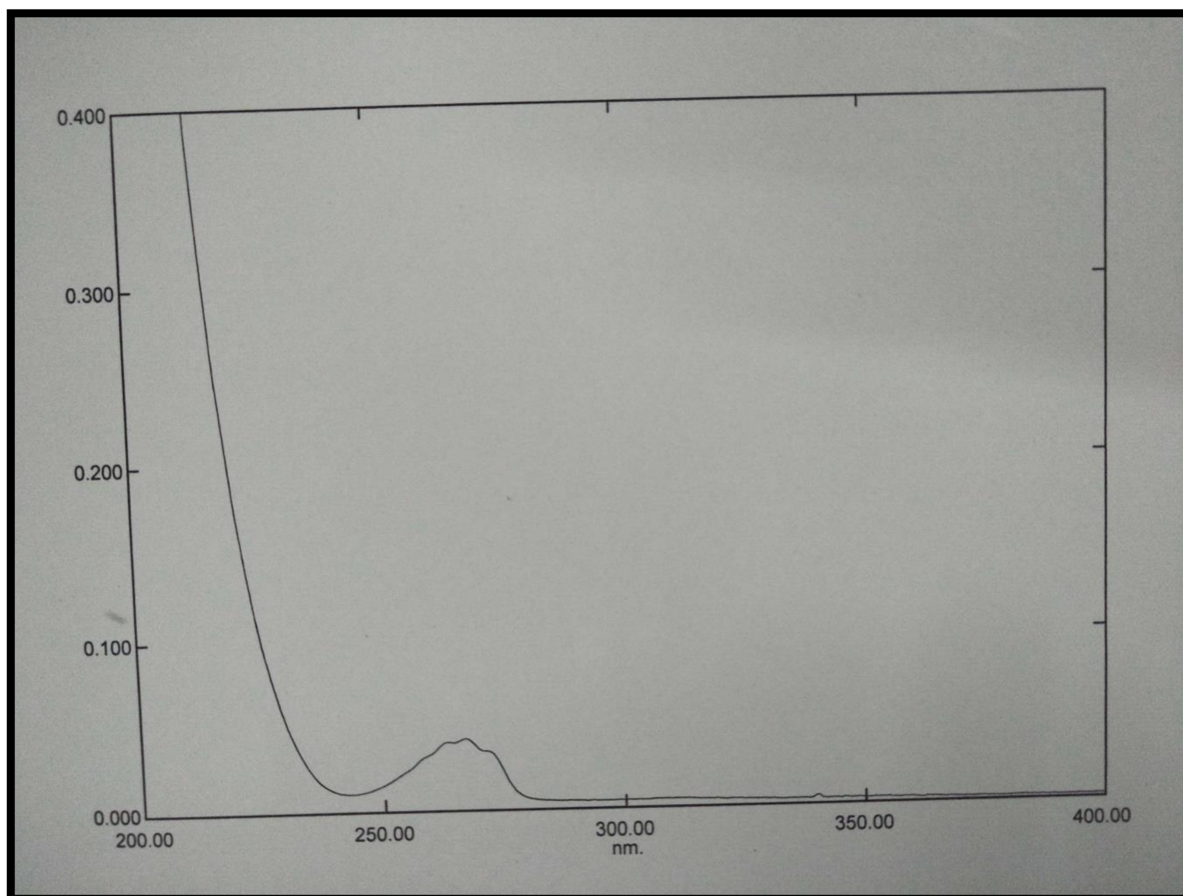


FIGURE 4b- CALIBRATION OF SITAGLIPTIN PHOSPHATE IN PHOSPHATE BUFFER P^H 7.2

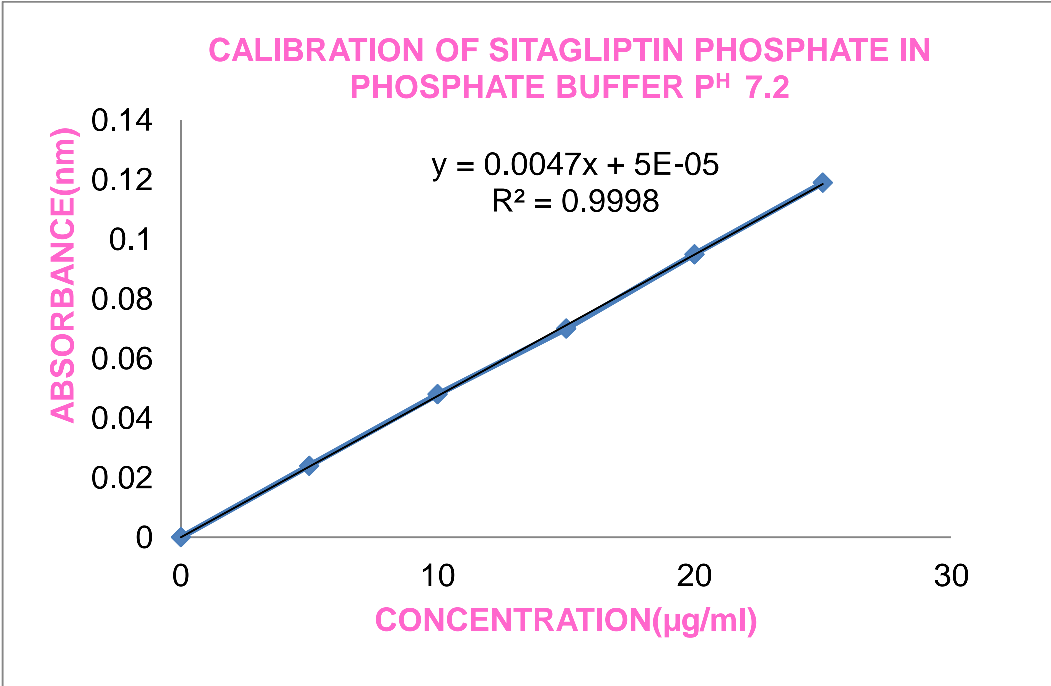


FIGURE-4c DETERMINATION OF λmax FOR SITAGLIPTIN PHOSPHATE USING 0.1N HCL

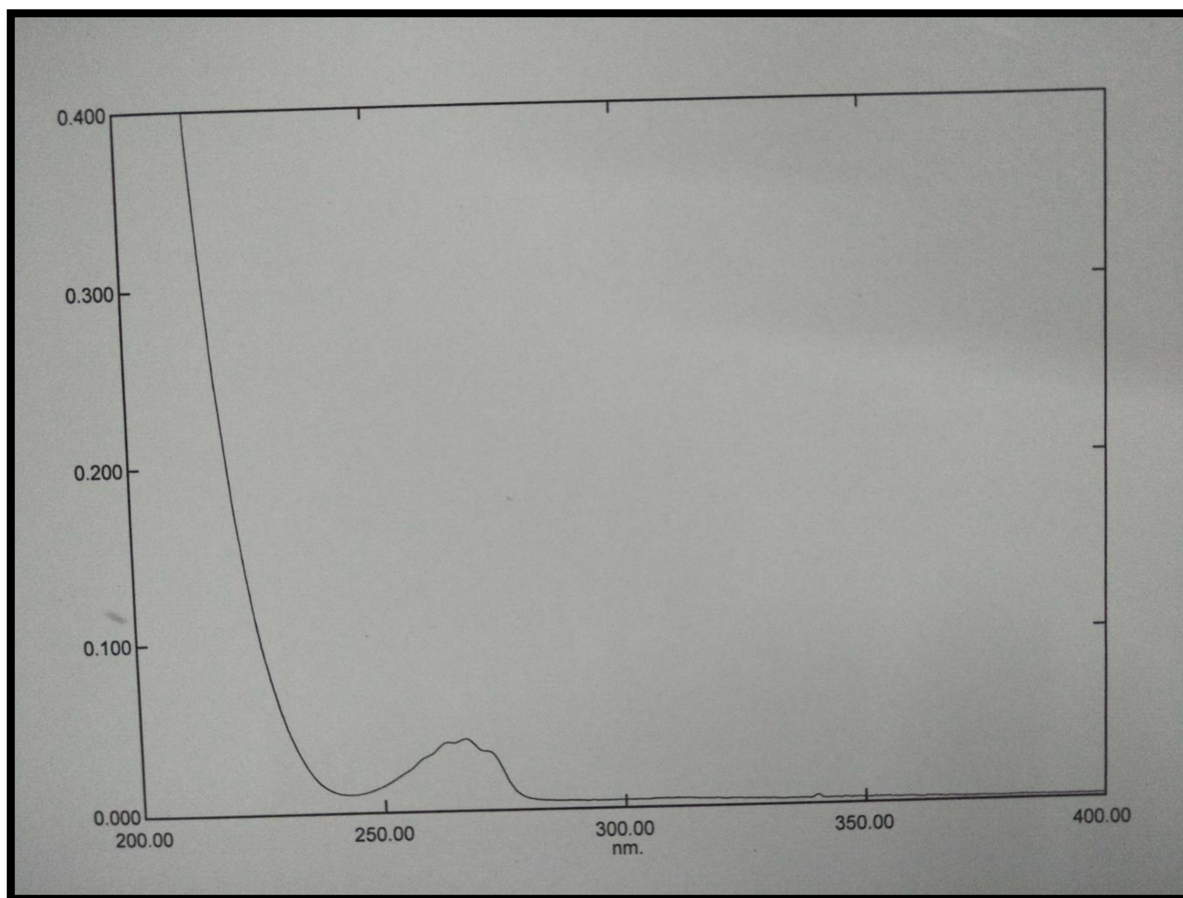


FIGURE 4d- CALIBRATION OF SITAGLIPTIN PHOSPHATE IN 0.1N HCL

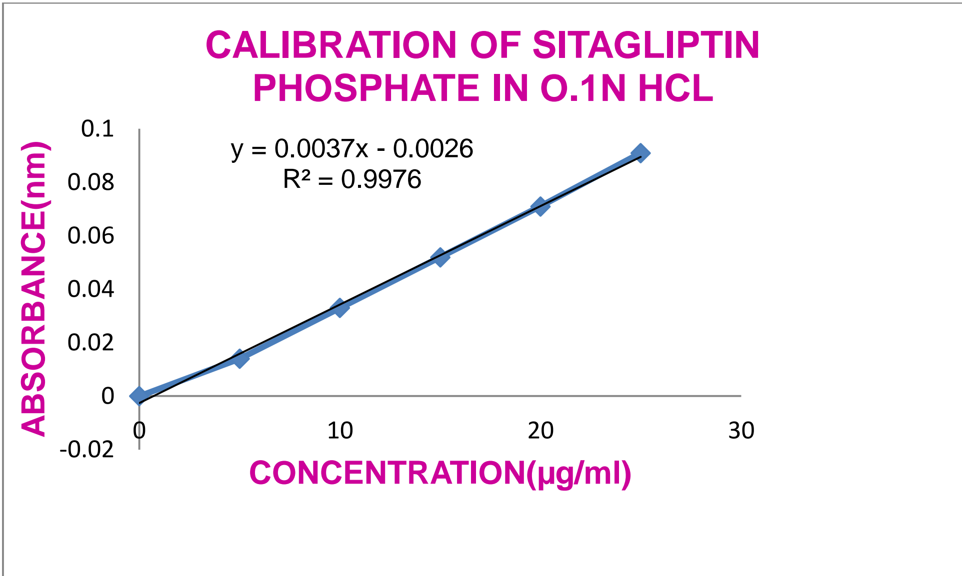


FIGURE -5a IR SPECTRA OF SITAGLIPTIN PHOSPHATE

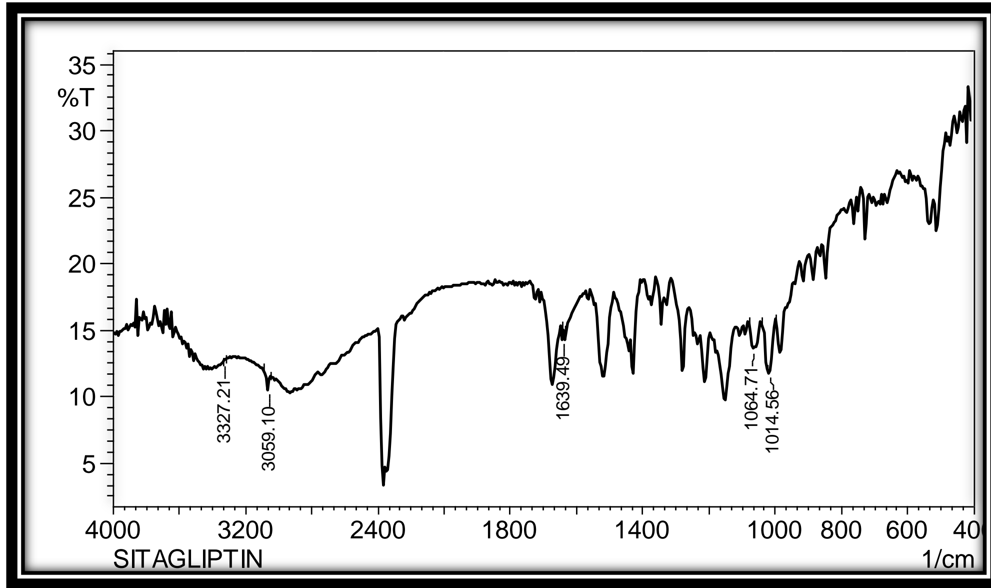


FIGURE 5b – IR SPECTRA OF EUDRAGIR RS 100

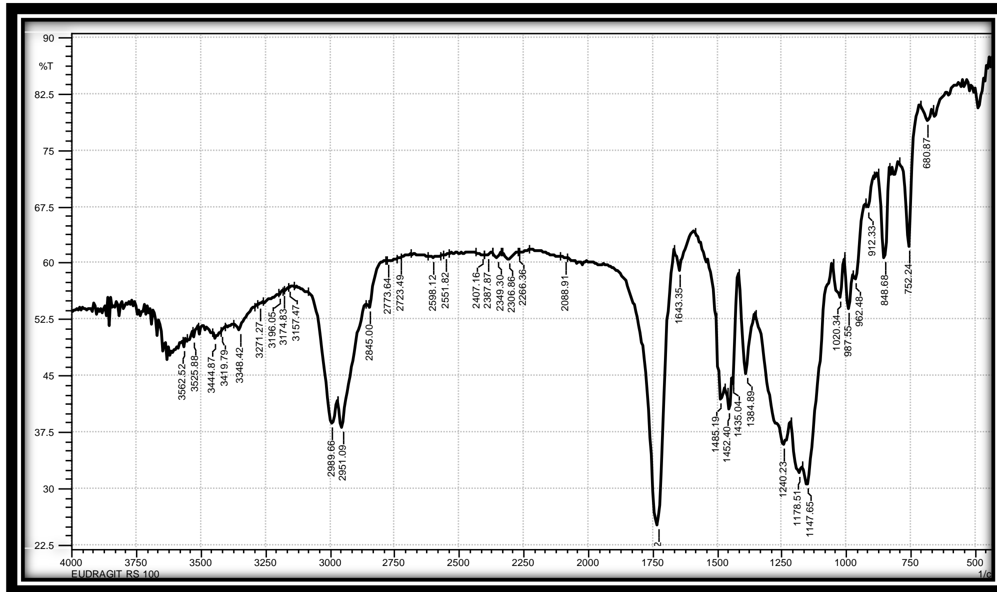


FIGURE 5c- IR SPECTRA OF SITAGLIPTIN+EUDRAGIT RS 100+ HPMC E15+ CHITOSAN+CARBOPOL 934

%T

20
17.5
15
12.5
10
7.5
5
2.5
0

4000 3200 2400 1800 1400 1000 800 600 400

SITAGLIPTIN WITH POLYMER MIXTURE 1/cm

FIGURE 5d- IR SPECTRA OF SITAGLIPTIN FORMULATION (BEST)

%T

37.5
35
32.5
30
27.5
25
22.5
20
17.5

4000 3200 2400 1800 1400 1000 800 600 400

SITAGLIPTIN FORMULATION 1/cm

FIGURE 6a – DSC THERMOGRAM OF SITAGLIPTIN PHOSPHATE

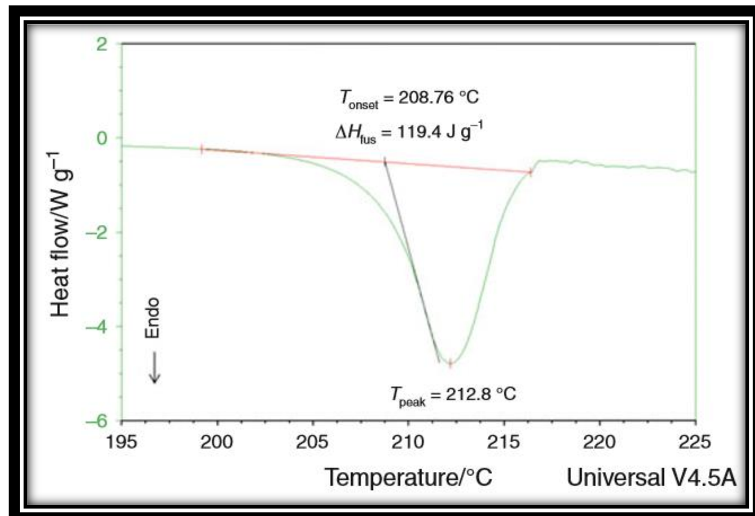


FIGURE 6b- DSC THERMOGRAM OF SITAGLIPTIN BEST FORMULATION

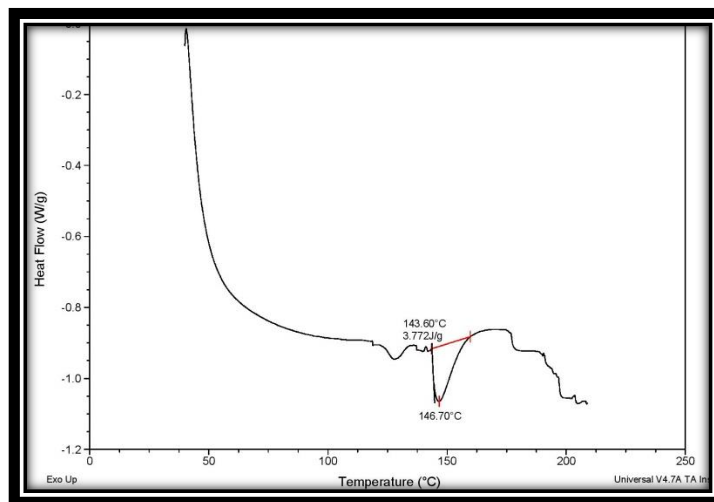


FIGURE 7- PERCENTAGE YIELD OF SITAGLIPTIN MICROSPHERES

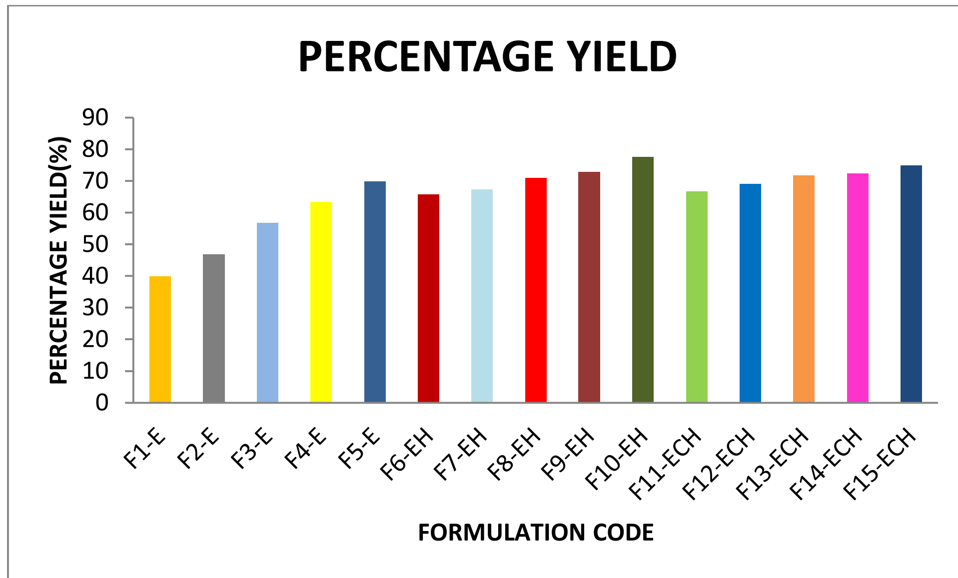


FIGURE -8 THEORETICAL DRUG LOADING FOR SITAGLIPTIN MICROSPHERES

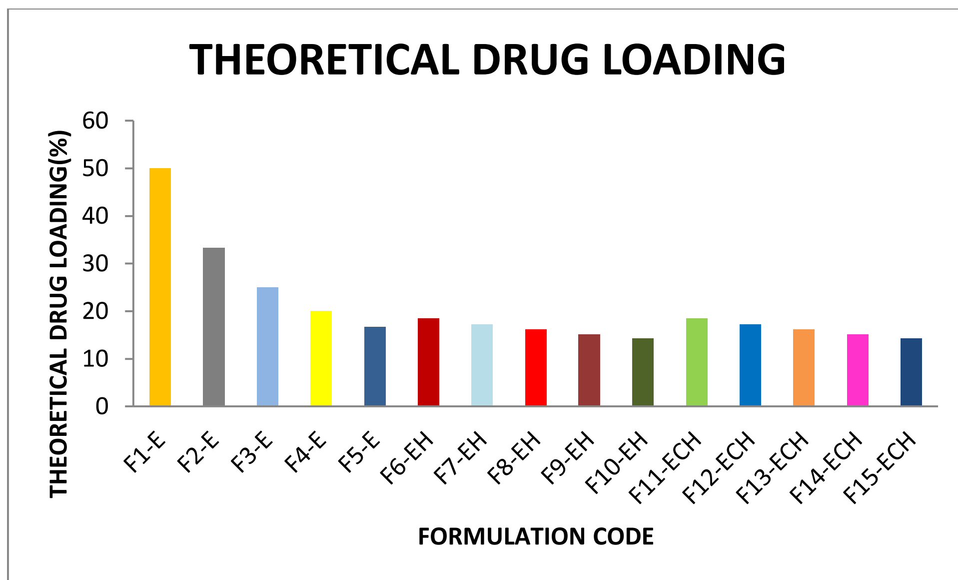


FIGURE -9 EXPERIMENTAL DRUG LOADING FOR SITAGLIPTIN MICROSPHERES

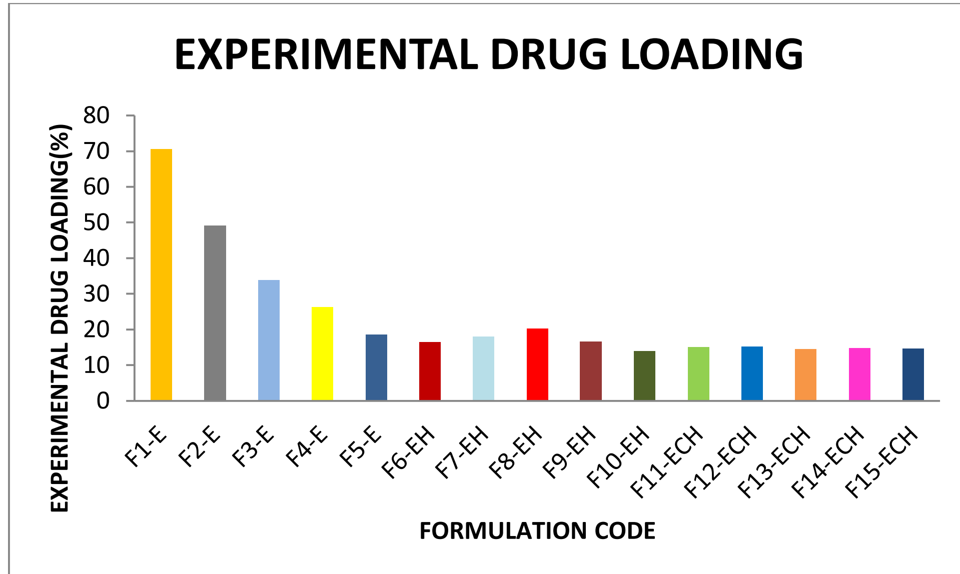


FIGURE-10 DRUG CONTENT FOR SITAGLIPTIN MICROSPHERES

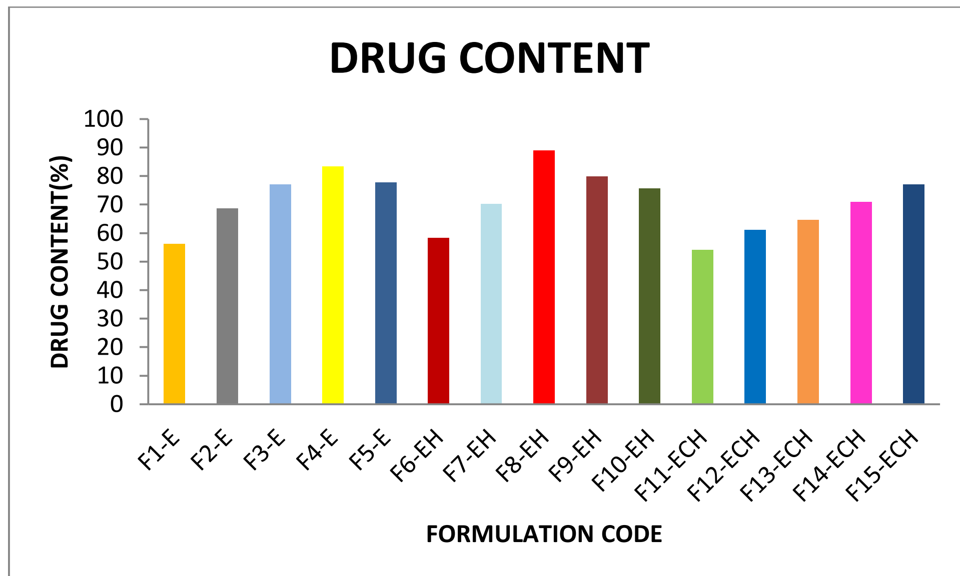


FIGURE-11 ENCAPSULATION EFFICIENCY FOR SITAGLIPTIN MICROSPHERES

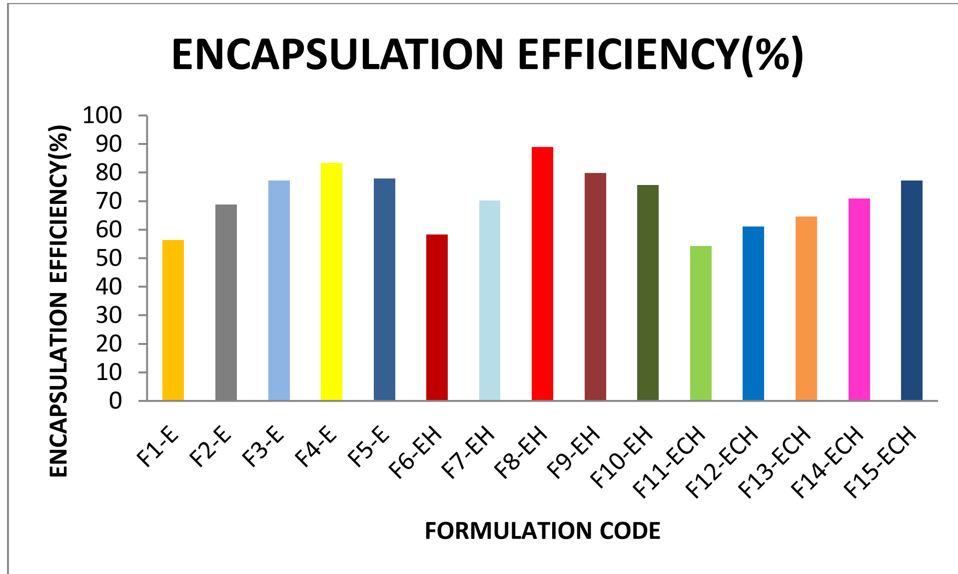


FIGURE-12 MEAN PARTICLE SIZE FOR SITAGLIPTIN MICROSPHERES

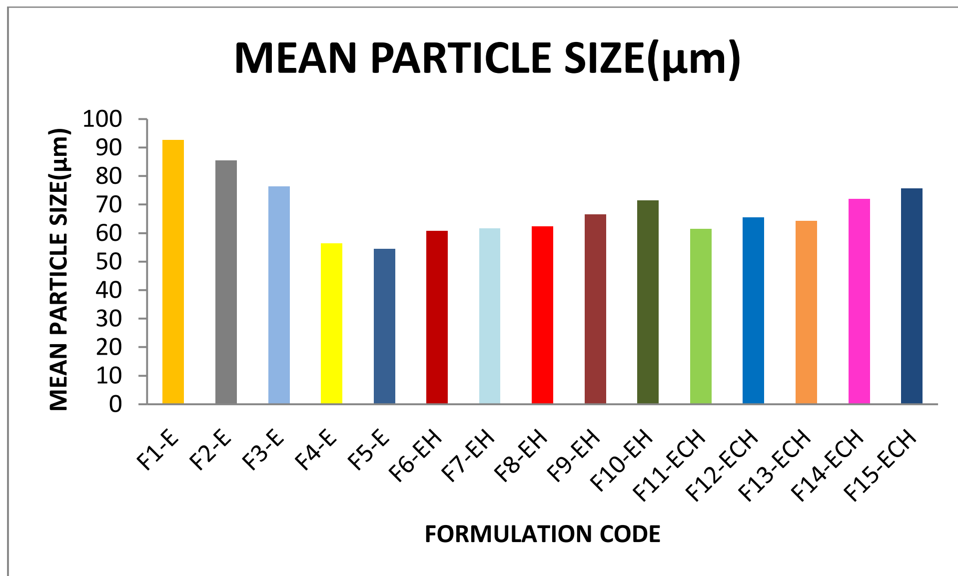


FIGURE-13a COMPARISON OF INVITRO RELEASE PROFILE OF SITAGLIPTIN PHOSPHATE MICROSPHERES CONTAINING EUDRAGIT RS 100 AT DIFFERENT RATIOS

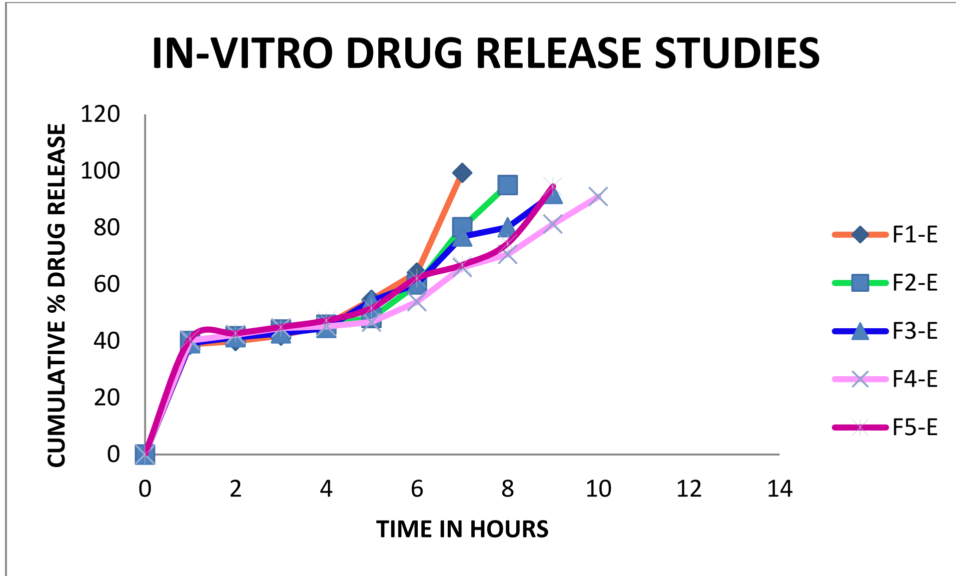


FIGURE-13b COMPARISON OF INVITRO RELEASE PROFILE OF SITAGLIPTIN PHOSPHATE MICROSPHERES CONTAINING EUDRAGIT RS 100 WITH HPMC-E15 AT DIFFERENT RATIOS

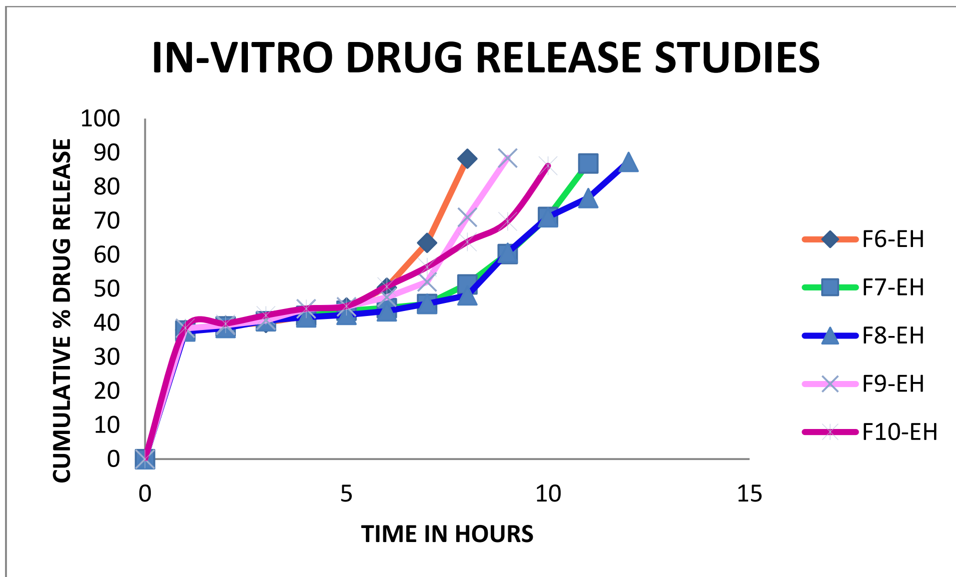


FIGURE-13c COMPARISON OF INVITRO RELEASE PROFILE OF SITAGLIPTIN PHOSPHATE MICROSPHERES CONTAINING EUDRAGIT RS 100 WITH CHITOSAN AT DIFFERENT RATIOS

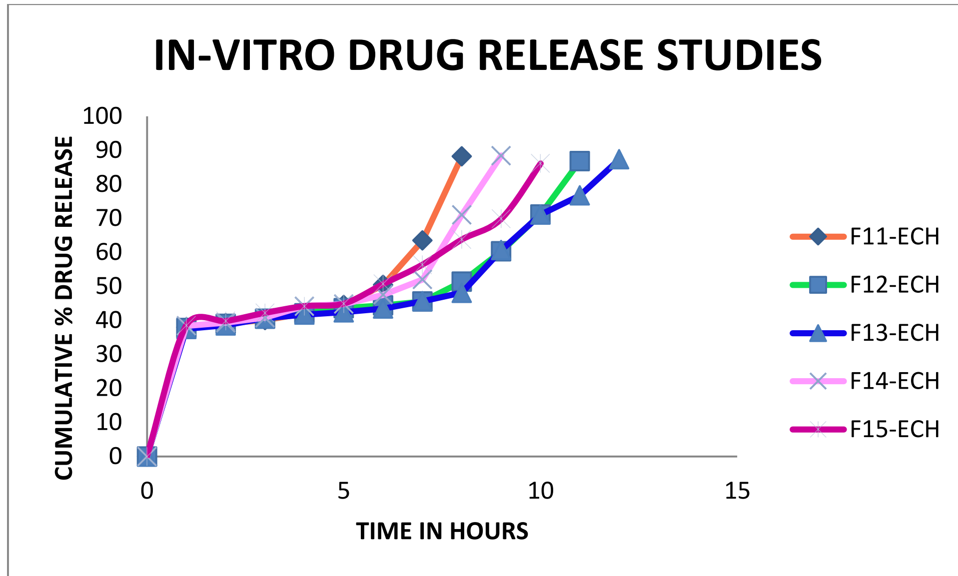


FIGURE-13d COMPARISON OF INVITRO RELEASE PROFILE OF SITAGLIPTIN PHOSPHATE FOR SELECTION OF BEST FORMULATION

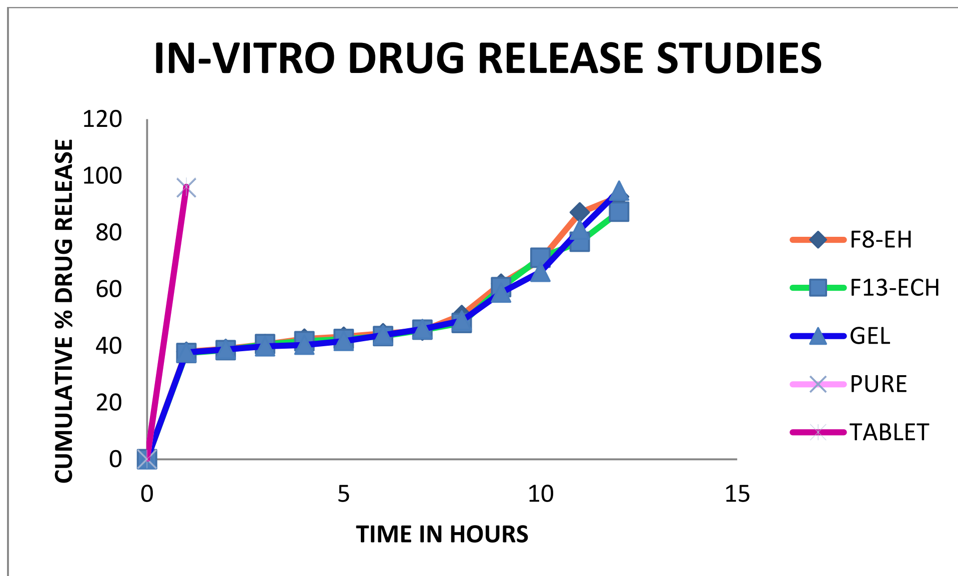


FIGURE-14a COMPARISON OF IN-VITRO ZERO ORDER RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 AT DIFFERENT RATIOS

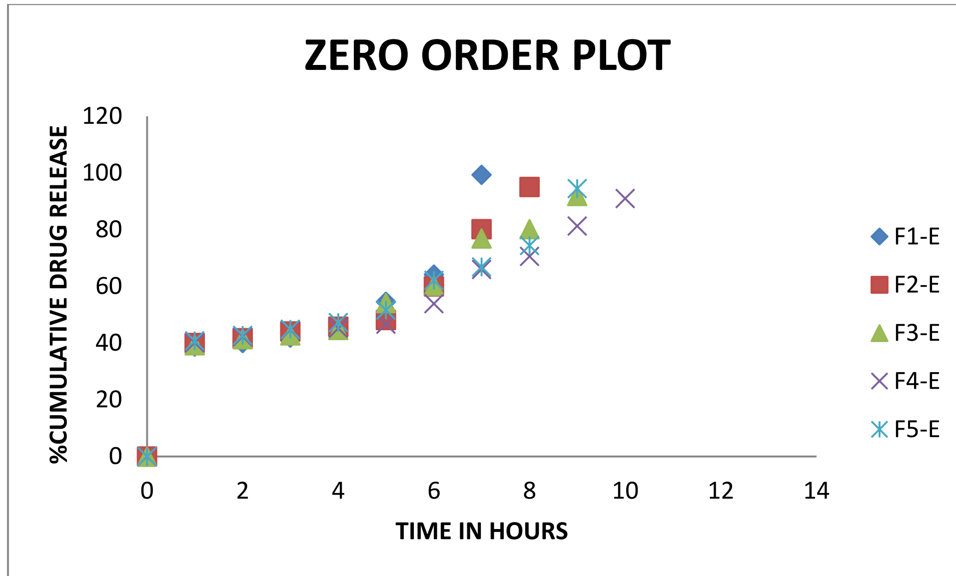


FIGURE-14b COMPARISON OF IN-VITRO ZERO ORDER RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH HPMC E-15 RATIOS

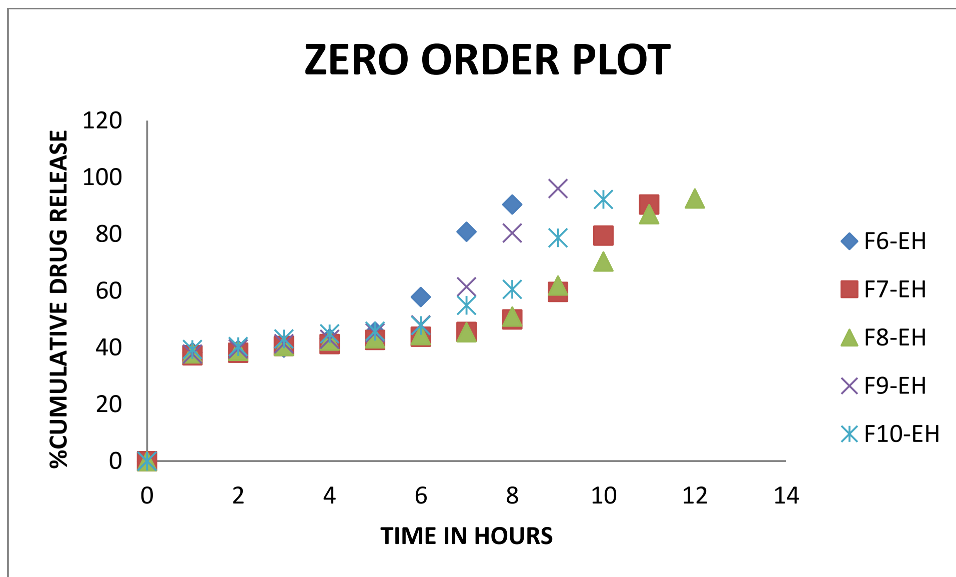


FIGURE-14c COMPARISON OF IN-VITRO ZERO ORDER RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH CHITOSAN RATIOS

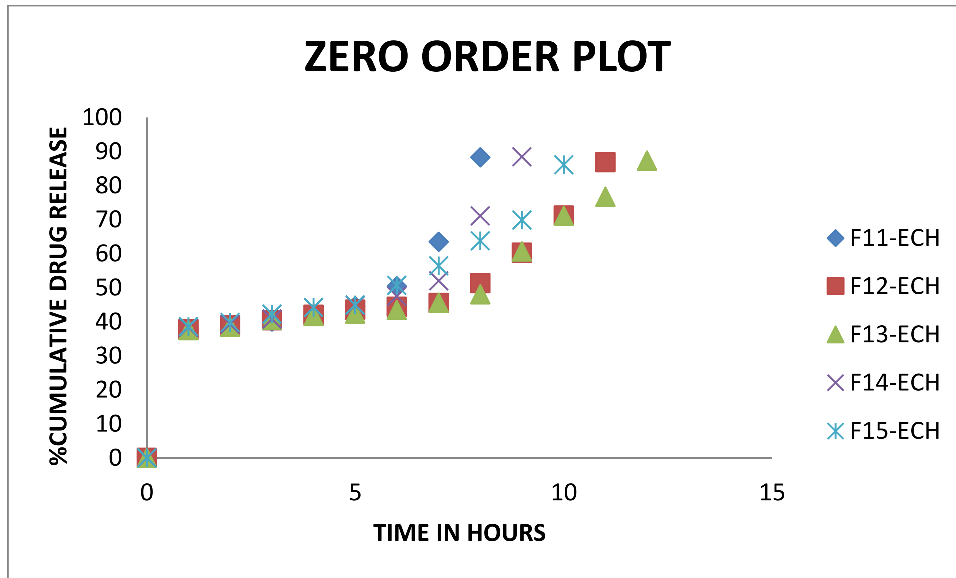


FIGURE-15a COMPARISON OF IN-VITRO FIRST ORDER RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 AT DIFFERENT RATIOS

FIGURE-15b COMPARISON OF IN-VITRO FIRST ORDER RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH HPMC E-15 RATIOS

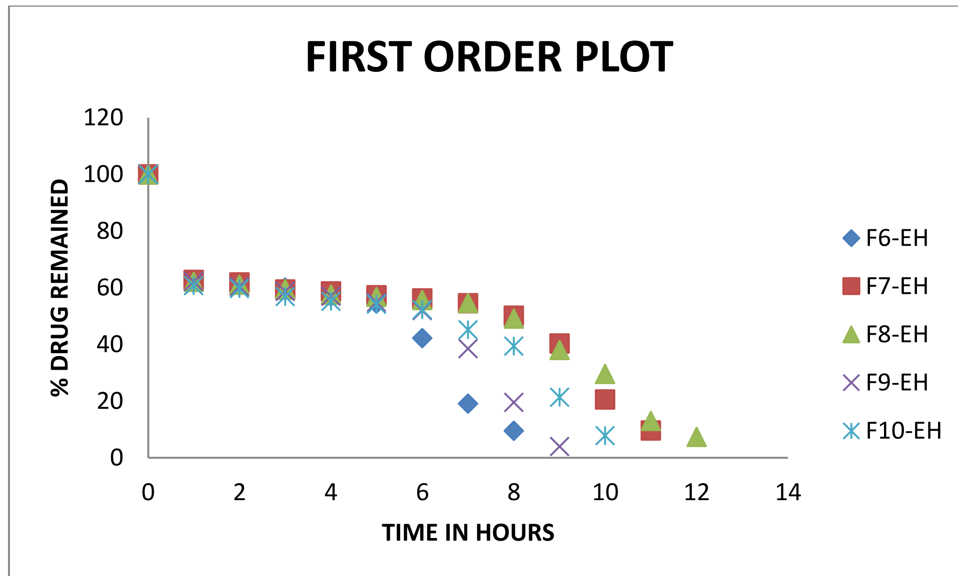


FIGURE-15c COMPARISON OF IN-VITRO FIRST ORDER RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH CHITOSAN RATIOS

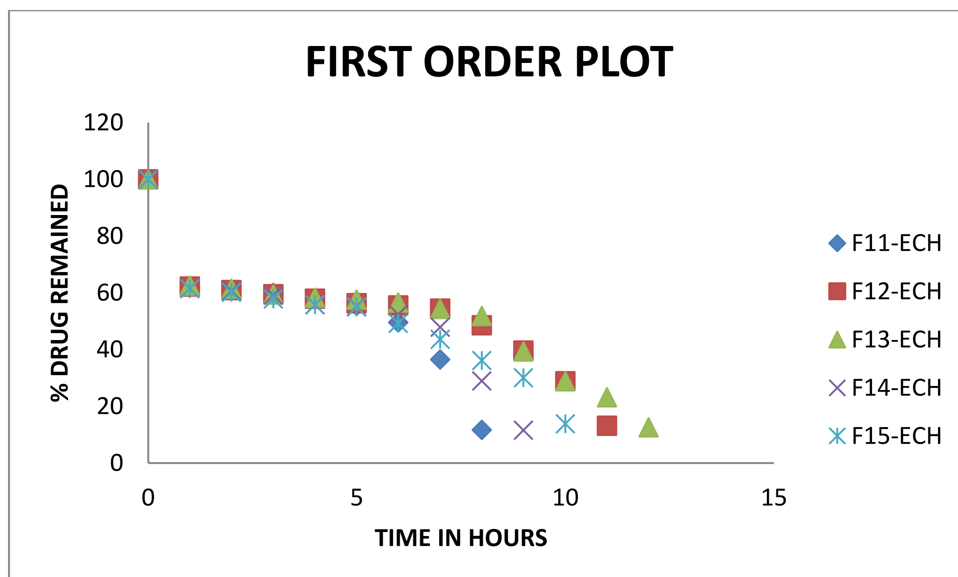


FIGURE-16a COMPARISON OF IN-VITRO HIGUCHI MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 AT DIFFERENT RATIOS

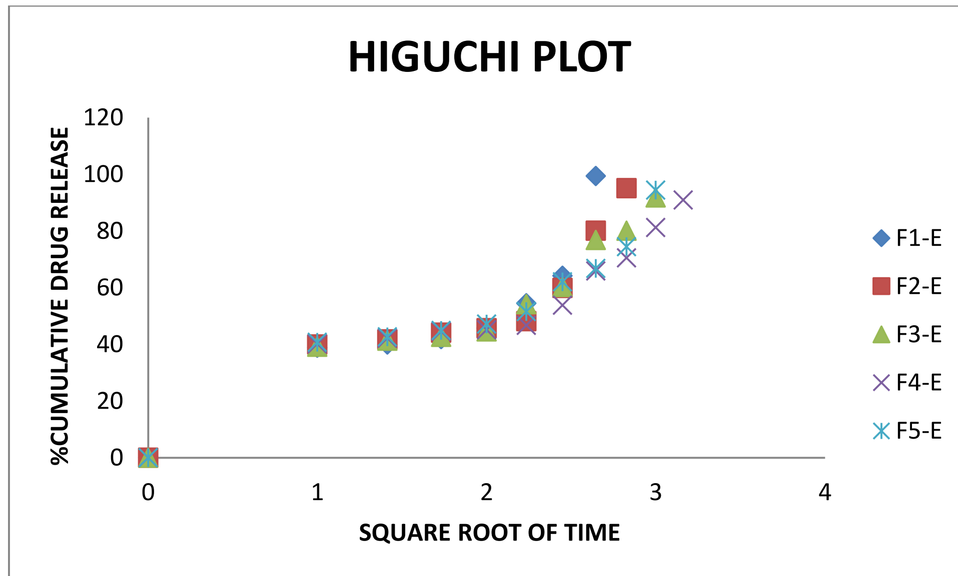


FIGURE-16b COMPARISON OF IN-VITRO HIGUCHI MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH HPMC E-15 RATIOS

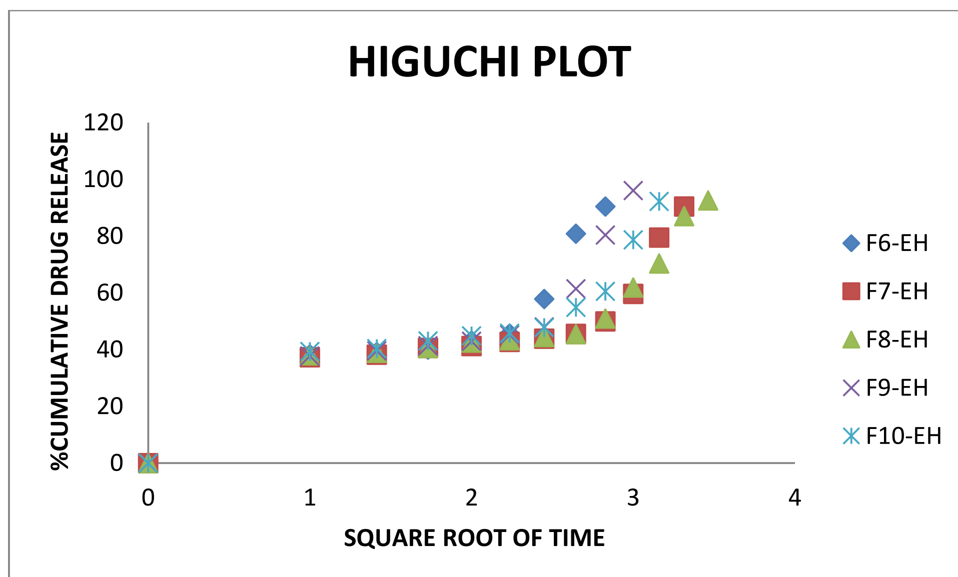


FIGURE-16c COMPARISON OF IN-VITRO HIGUCHI MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH CHITOSAN RATIOS

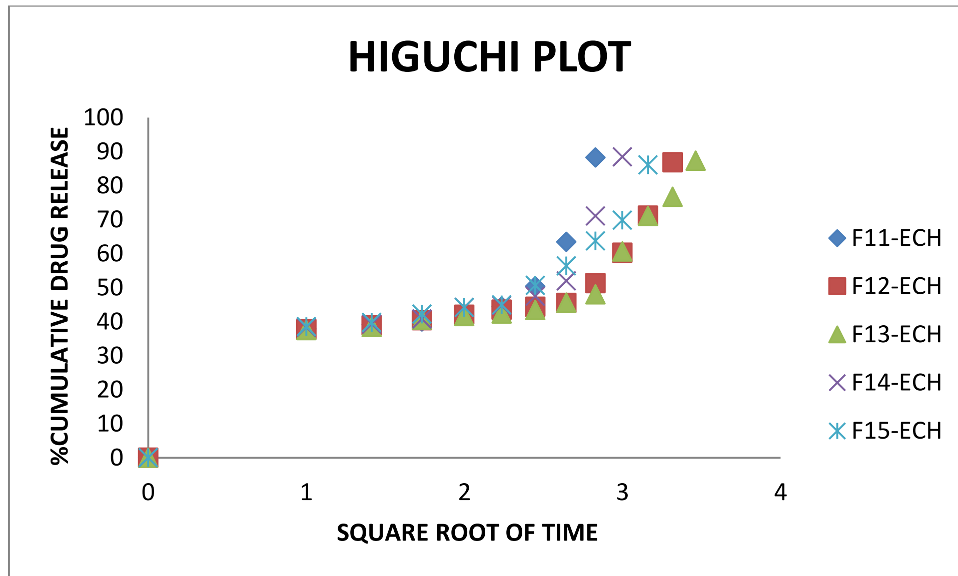


FIGURE-17a COMPARISON OF IN-VITRO KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 AT DIFFERENT RATIOS

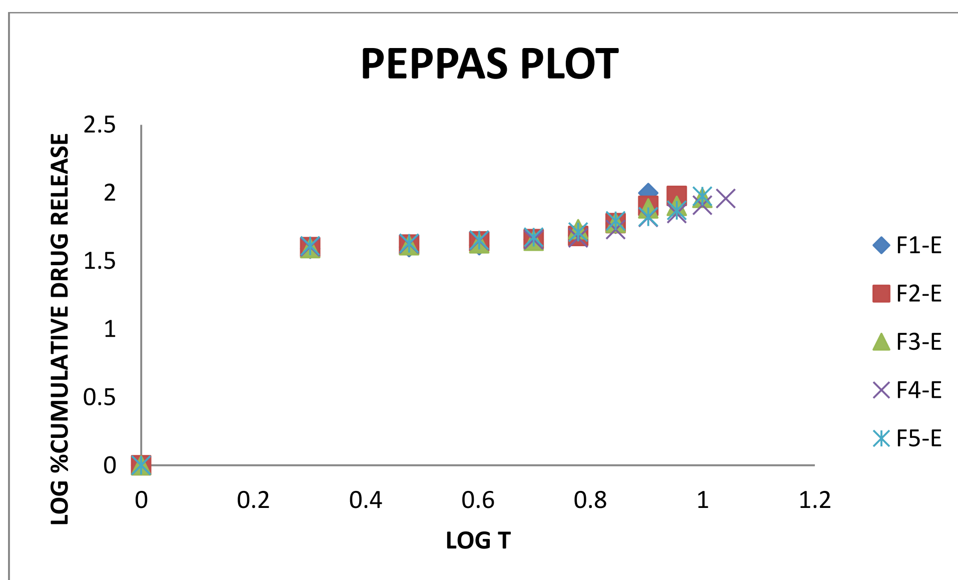


FIGURE-17b COMPARISON OF IN-VITRO KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH HPMC E-15 RATIOS

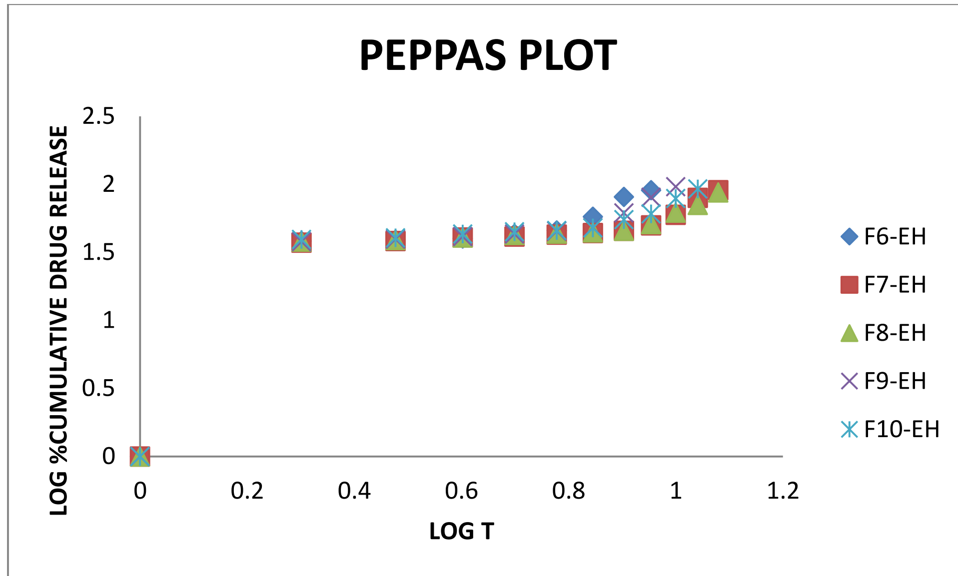


FIGURE-17c COMPARISON OF IN-VITRO KORSMEYER PEPPAS MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH CHITOSAN RATIOS

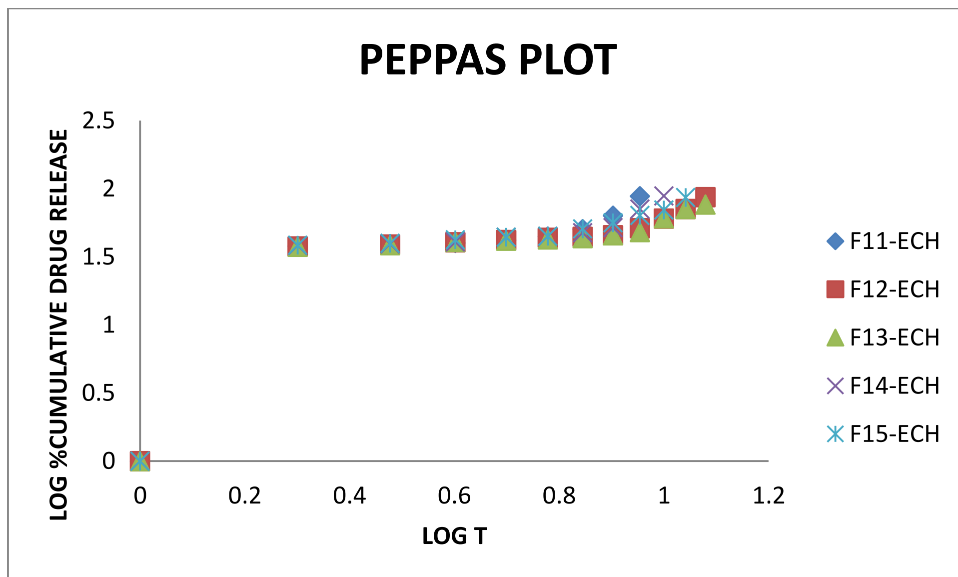


FIGURE-18a COMPARISON OF IN-VITRO HIXSON CROWEL MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 AT DIFFERENT RATIOS

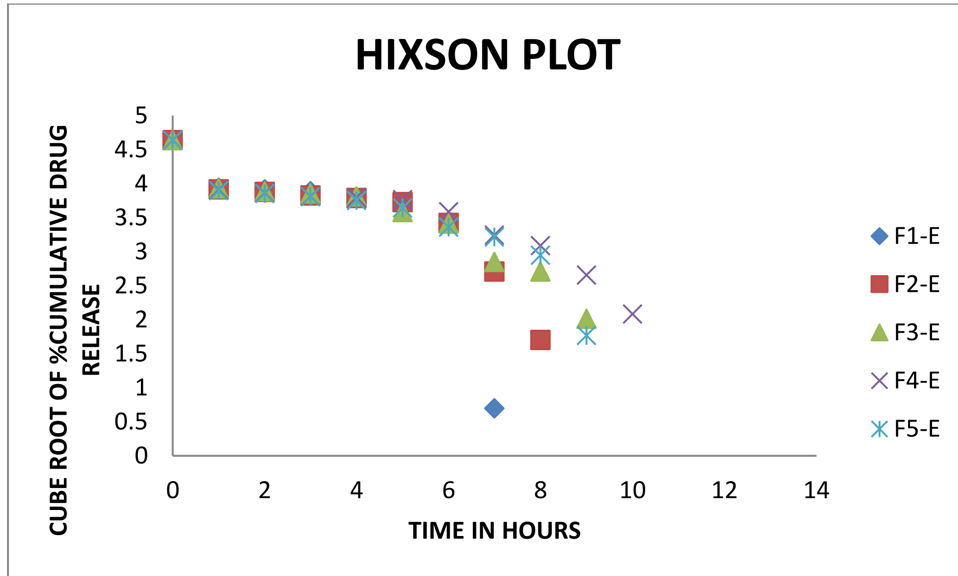


FIGURE-18b COMPARISON OF IN-VITRO KORSMEYER HIXSON CROWEL MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH HPMC E-15 RATIOS

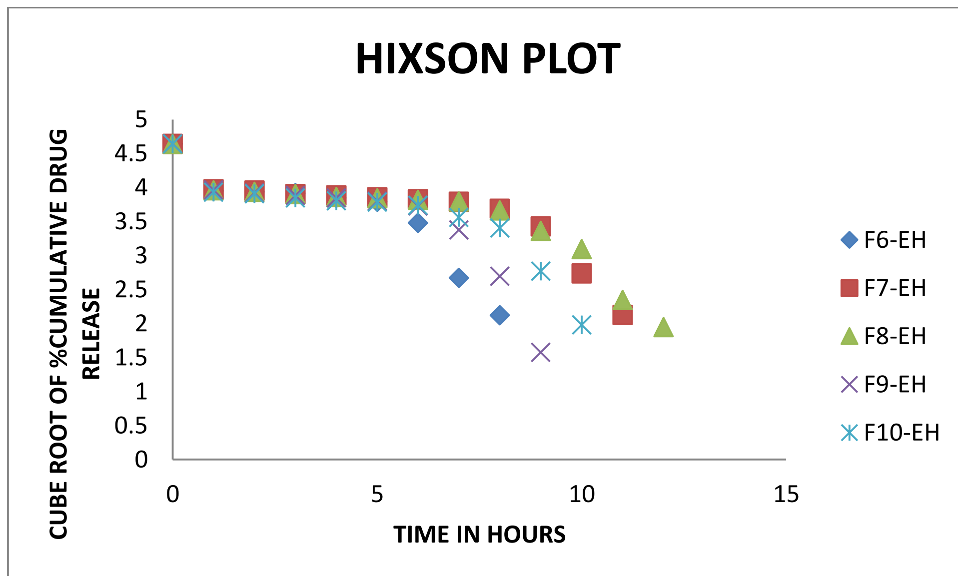


FIGURE-18c COMPARISON OF IN-VITRO HIXSON CROWEL MODEL RELEASE KINETICS OF SITAGLIPTIN PHOSPHATE MICROSPHERES BY USING EUDRAGIT RS 100 WITH CHITOSAN RATIOS

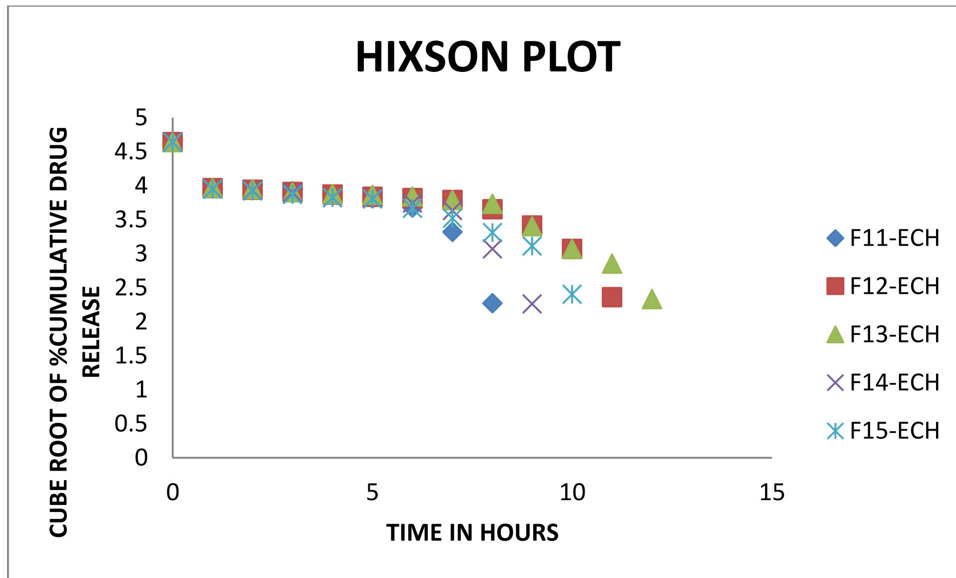


FIGURE-19 SCANNING ELECTRON MICROSCOPY OF SITAGLIPTIN MICROSPHERES

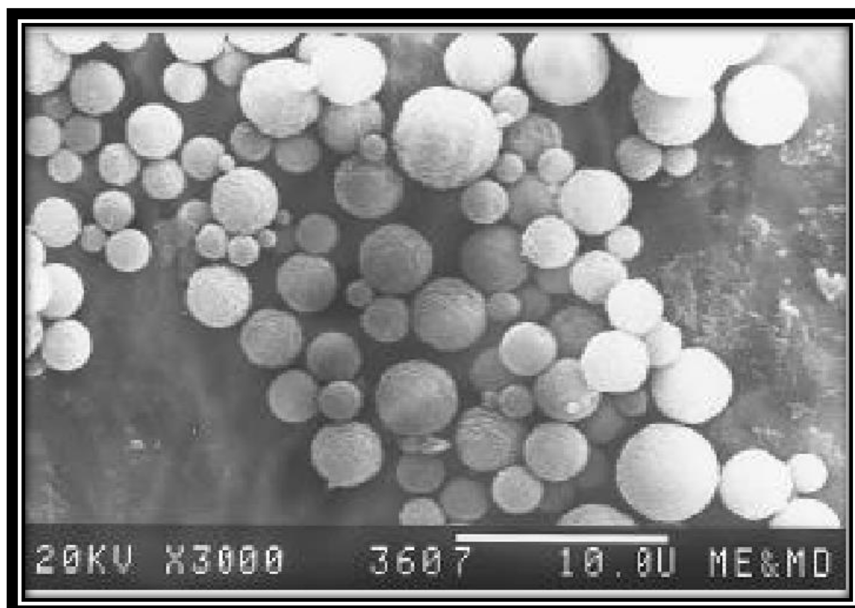


FIGURE 20-a STABILITY STUDIES FOR SITAGLIPTIN PHOSPHATE MICROSPHERES

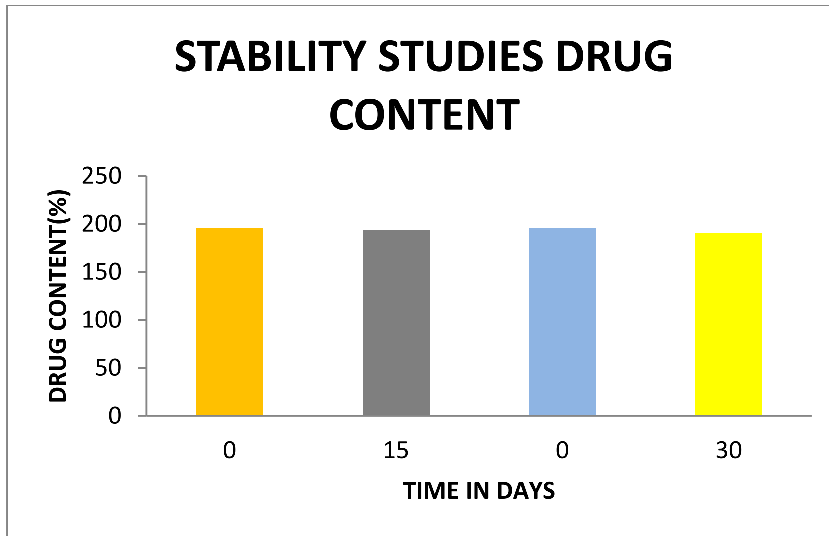


FIGURE 20-b STABILITY STUDIES FOR SITAGLIPTIN PHOSPHATE MICROSPHERES

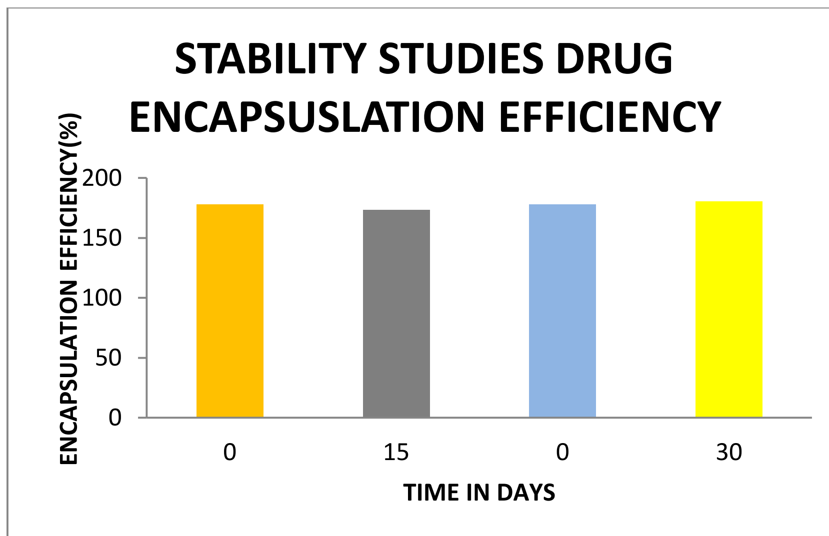
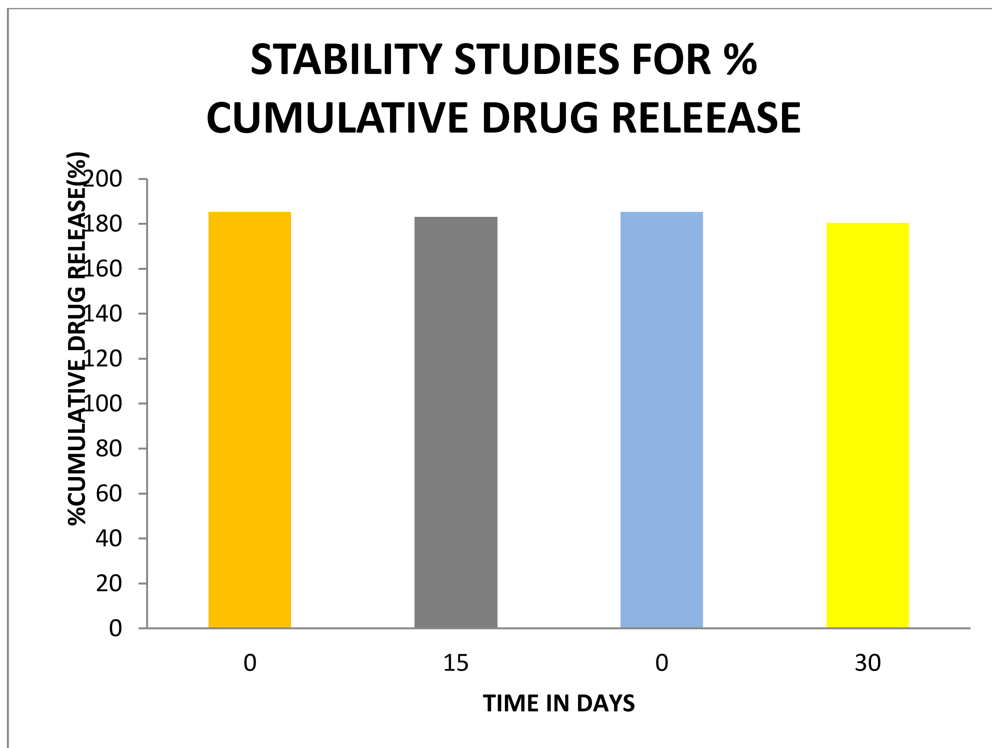


FIGURE 20-C STABILITY STUDIES FOR SITAGLIPTIN PHOSPHATE MICROSPHERES



CHAPTER – XI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

- In this present study was to formulate and evaluate sustained release microsphere gel loaded sitagliptin phosphate in order to maintain a sustained drug concentration in serum for longer period of time, which may result in enhanced absorption and thereby improved bioavailability.
- The results of compatibility studies by infrared spectroscopy and differential scanning calorimetry showed no interaction between the drug and polymers.
- The microgel of sustained release microspheres of sitagliptin phosphate were successfully prepared by non-aqueous solvent evaporation technique.
- All formulations F1 to F15 micropheres were evaluated for particle size analysis mean particle size range 54.51 to 92.62 μm.
- Mean particle size range of sitagliptin microspheres were in the range of suitable size range
- The F8-EH shows the maximum drug content values of 97.92% Percentage Encapsulation efficiency of the F8 – 88.88 %. As the polymer concentration was increased the drug entrapment efficiency % was increased due to increase in the viscosity of the solution. The present investigation state that if the drugs are soluble in the solvent system, it results in high drug encapsulation efficiency than that of dispersed in the solvent system. The elimination of the drugs from the prepared microspheres highly dependent on the concentration of the polymer used, as the amount of the polymer increased the encapsulation efficiency of the microsphere increased because of the good matrix formation.
- Scanning electron microphotographs showed that the microspheres were spherical with a smooth to rough surface.

- In-vitro release study of all the formulations were showed a sustained drug release with increase in concentration of different rate retardant polymers (EUDRAGIT RS 100, HPMC E15, CHITOSAN). Dissolution rate of all the formulations were sustained when compared to pure drug.
- The selected best formulation was formulated into gel using carbopol 934 by loading sitagliptin phosphate microspheres in carbomer gel.
- The dissolution study was carried out in phosphate buffer pH 7.2 for 12 hours. The formulations shows sustained release of drugs up to 12 hours and all formulations showed more than 90% drug release.

CONCLUSION

Sustained release microspheres of sitagliptin phosphate prepared with the rate retardant polymers successfully by the non-aqueous solvent evaporation technique.

From the results it was observed that drug: polymer ratio influences the particle size, drug content, encapsulation efficiency and in-vitro drug release of sustained release microspheres.

The formulated microspheres showed good drug-loading. The gel formulation, which consisted of drug-loaded sitagliptin microspheres, showed sustained release of for 12 h, thus indicating their suitability for the sustained delivery of the drugs for the treatment of DM. However, further studies, including clinical tests are required to confirm the gel’s therapeutic efficacy.

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Thank you!