FORMULATION AND EVALUATION OF LOVASTATIN LOADED NANOSPONGES FOR THE TREATMENT OF HYPERLIPIDEMIA

A Dissertation submitted to

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

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in partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
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Submitted by

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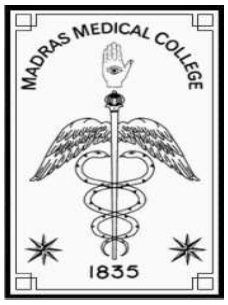

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Place: Chennai - 03

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LIST OF ABBREVIATIONS AND SYMBOLS

S.NO	SYMBOLS	ABBREVIATIONS
1	NDDS	Novel Drug Delivery System
2	NPDDS	Nanoparticles Drug Delivery System
3	NSs	Nanosponges
4	BCS	Biopharmaceutical Classification System
5	RES	Reticuloendothelial System
6	RTI	Respiratory Tract Infection
7	HBV	Hepatitis B Virus
9	HSV	Herpes Simpler Virus
10	EC	Ethyl Cellulose
11	EUD	Eudragit RS 100
12	PVA	Poly Vinyl Alcohol
13	PMMA	Poly methyl methacrylate
14	HPMC	Hydroxypropyle Methylcellulose
15	LOV	Lovastatin
16	SEM	Scanning Electron Microscopy
17	ZP	Zeta Potential
18	DSC	Differential Scanning Colorimetry
19	FT-IR	Fourier Transform Infra Red
20	XRPD	X-Ray Powder Diffraction
21	XRD	X-Ray Diffraction
22	PDI	Polydispersity index
23	HPLC	High Pressure Liquid Chromatography
24	HPTLC	High Pressure Thin Layer Chromatography
25	NMR	Nuclear Magnetic Resonance
26	UV	Ultraviolet Spectroscopy
27	MRSA	Methicillin Resistant Staphylococcus Aureus
28	SNEDDS	Self-Nanoemulsifying Drug Delivery System
29	SMEDDS	Self-Microemulsifying Drug Delivery System
30	GRDDS	Gastro Retentive Drug Delivery System

S.NO	SYMBOLS	ABBREVIATIONS
31	C-NSs	Capsule in Nanosponges
32	ER	Extended Release
33	IR	Immediate Release
34	CVD	Cardio Vascular Diseases
35	HMG-CoA	3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A
36	ASCVD	Atherosclerotic Cardiovascular Disease
37	LDL-C	Low Density Lipoprotein Cholesterol
38	FH	Familial Hypercholesterolemia
39	LFT	Liver Function Test
40	F. Code	Formulation Code
41	ICH	International conference on Harmonization
42	NC	No Change
43	RH	Relative Humidity
44	SD	Standard Deviation
45	rpm	Revolutions Per Minute
46	mg	Milligram
47	g	Gram
48	ml	Milliliter
49	mV	Millivolts
50	µm	Micrometer
51	%	Percentage
52	%w/w	Percentage weight by weight
53	min	Minute
54	mm	Millimeter
55	hrs	Hours
56	µg	Microgram
57	nm	Nanometre

INTRODUCTION

1. INTRODUCTION

1.1 DRUG DELIVERY SYSTEM

A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. Drug delivery system is an interface between the patient and the drug.[1]

Drug delivery is a concept integrated with dosage form and route of administration of pharmaceutical products. The technologies involved in the formulation of pharmaceutical products modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms. Most common routes of administration are noninvasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes.[2]

An ideal drug-delivery system possesses two elements: the ability to target and to control the drug release. Targeting will ensure high efficiency of the drug and reduce the side effects, especially when dealing with drugs that are presumed to kill cancer cells but can also kill healthy cells when delivered to them. The reduction or prevention of side effects can also be achieved by controlled release. NPDDS provide a better penetration of the particles inside the body as their size allows delivery via intravenous injection or other routes. The nanoscale size of these particulate systems also minimizes the irritant reactions at the injection site.[3]

REASON FOR DEVELOPMENT OF DRUG DELIVERY SYSTEM[4, 5]

Over the past three decades, a great deal of attention has been focused on the development of new drug delivery system. There are many reasons for the interest into this drug delivery system. They are

- As bringing new drug entities into the market is an expensive and time consuming process, so improving safety efficacy ratio of "old" drugs to development of new drug delivery is profitable.
- Delivering drug at controlled rate, slow delivery, targeted delivery are other very attractive methods.
- New system is needed to deliver novel, genetically engineered pharmaceuticals such as protein and peptides to their sites of action without biological inactivation.
- To improve therapeutically efficacy and safety of conventional drug, the size and frequency of dose is reduced.
- Two important features are important while developing a drug delivery system. i.e. It should deliver drug at a rate dictated by needs of body over the entire period of treatment and the drug should solely reach the site of action.

DRAWBACKS OF CONVENTIONAL DOSAGE FORMS[6]

An ideal dosage regimen in the drug therapy of any disease is one which immediately attains the desired therapeutic concentration of drug in plasma and maintains it constant for the entire duration of treatment. This is possible through the administration of drug delivery system in a particular dose and at particular frequency. The frequency of administration or dose interval of any drugs depends upon its half life or mean residence time and its therapeutic index. In most cases, dosing interval is much shorter than the half life of the drug, resulting in number of limitations associated with such a conventional dosage form which are,

- Drugs with short half-life require frequent administration, which increases chances of missing the dose of drug leading to poor patient compliance.
- A typical peak alley plasma concentration-time profile is obtained which makes attainment of steady state condition very difficult.
- The unavoidable fluctuations in the drug concentration may lead to under medication or over medication as the steady state concentration values fall or rise beyond the therapeutic range which may lead to precipitation of adverse effect.
- Drug accumulation may occur in case of the frequent administration of the drugs.

To overcome the above drawbacks, drug delivery system capable of controlling the rate of drug delivery, sustain the duration of therapeutic action or targeting the drug to a particular tissue was developed.

1.2 NOVEL DRUG DELIVERY SYSTEM

Novel drug delivery system (NDDS) is advanced drug delivery system which improves drug potency, control drug release to give a sustained therapeutic effect, provide greater safety; finally to target a drug specifically to a desired tissue. The basic goal of novel drug delivery system is to achieve a steady state blood or tissue level that is therapeutically effective and non-toxic for extended period of time.NDDS are being developed rapidly, so as to overcome the limitation of conventional drug delivery.[7]

This idealized objectives witch to the two main aspects they are as follows

I. **Spatial Drug Delivery:**
Targeting a drug to a particular organ or tissue.

II. **Temporal Drug Delivery:**
The drug delivery rate to the target tissue is controlled.

NOVEL DRUG DELIVERY SYSTEM CAN BE DIVIDED INTO 2 SYSTEMS

- Sustained release drug delivery system
- Controlled release drug delivery system

SUSTAINED RELEASE DRUG DELIVERY SYSTEM

Any drug or dosage form modification that prolongs the therapeutic activity of the drug. The release of the drug is retarded for a delayed and prolonged period of time in the systemic circulation. Sustained release formulation maintains a uniform blood level of drug with better patient compliance as well as increased efficacy of drug. Sustained release tablets are generally taken once or twice a day during a course of treatment whereas in conventional dosage forms there is need to take 3-4 times dosage in a day to achieve the same therapeutic action.

CONTROLLED RELEASE DRUG DELIVERY SYSTEM

The controlled release system is to deliver a constant supply of the active ingredient, usually at a zero-order rate, by continuously releasing, for a certain period of time, an amount of the drug equivalent to the eliminated by the body. An ideal

Controlled drug delivery system is the one, which delivers the drugs at a predetermined rate, locally or systematically, for a specific period of time. They are,

- ❖ Rate pre-programmed drug delivery system
- ❖ Activation modulated drug delivery system
- ❖ Feed- back regulated drug delivery system
- ❖ Site targeting drug delivery system

Merits of controlled drug delivery system

a) Improved patient compliance and convenience
b) Reduction in fluctuation in steady state levels
c) Increased safety margin of high potency drugs due to better control of plasma level.
d) Maximum utilization of drug enabling reduction in total amount of dose administered.
e) Reduced in health care cost through
   - ➢ Improved therapy
   - ➢ Shorten treatment period
   - ➢ Lower frequency of dosing
   - ➢ Reduction in personnel time to dispense, administer and monitor patients.

Demerits of CDDS:

a) Decreased systemic availability in comparison to immediate release conventional dosage forms, which may be due to
   - ➢ Incomplete release
   - ➢ Increased instability
   - ➢ Insufficient residence time for complete release
   - ➢ Site specific absorption
   - ➢ pH dependent stability etc.
   - ➢ Poor *in vitro-in vivo* correlation.

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

c) Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.
d) Reduced potential of dosage adjustment of drugs normally administered in varying strengths.[6,8]

1.3 ORAL CONTROLLED DRUG DELIVERY SYSTEM[9]

Oral route has been commonly adopted and most convenient route for the drug delivery. Oral route of administration has been received more attention in the pharmaceutical field because of the more flexibility in the designing of dosage form than drug delivery design for the other routes.

In the last two decades the drug delivery technology has been developed rapidly and many novel oral drug delivery systems has been invented. Despite tablets, capsules, suspension, emulsion, and solution, they are more superior to the conventional oral formulation. Because of their clinical advantages over immediate release pharmaceutical products containing the same drugs, sustained release system and controlled release drug delivery systems are developed which are interchangeable.

Some oral controlled drug delivery formulations

- Osmotic tablets
- Mucoadhesive tablets
- Matrix tablets
- Film coated tablets
- Enteric coated tablets
- Swellable tablets
- Floating capsules
- Microgranules and Spheroids
- Beads
- Pellets
- Microcapsules and microspheres

1.4 NANOTECHNOLOGY[10]

Nanotechnology can be defined as the science and engineering involved in the design characterization, production and applications of structures, devices and

systems by controlling shape and size at nanometer scale. Nanotechnology is providing solutions several pharmaceutical drug delivery issues

For over 20 years, researchers have appreciated the potential benefits of Nanotechnology in providing vast improvements in drug delivery and drug targeting. Improving delivery techniques that minimize toxicity and improve efficacy offers great potential benefits to patients, ands up new markets for pharmaceutical and drug delivery companies.

Need for Nanoparticle[11]

At present 95% of all new potential therapeutics have poor pharmacokinetic and biopharmaceutical properties. Therefore, there is a need to develop suitable drug delivery systems that distribute the therapeutically active drug molecule only to the site of action, without affecting healthy organs and tissues, also lowering doses required for efficacy as well as increasing the therapeutics indices and safety profiles of new therapeutics. Different reasons are,

1) Pharmaceutical

- Drug instability in conventional dosage form
- Solubility

2) Biopharmaceutical

-Low absorption
- High membrane bounding
- Biological instability

3) Pharmacokinetic/ Pharmacodynamic

- Short half life
- Large volume of distribution
- Low specificity

4) Clinical

- Low therapeutic index

1.5 DRUG DELIVERY CARRIERS[12]

In many cases, Colloidal drug carrier systems are used to improve stability of the drug either in biological fluids or in the formulation to develop extended-release

systems with targeting features to enhance therapeutic efficacy and reduce drug toxicity by modifying the distribution and controlling the disposition of the drug.

Biodegradable and non-biodegradable nano-carrier approaches could be used to improve the solubility issues of lipophillic drug molecules. Biodegradable nano-carriers (solid lipid nanoparticles, liposomes, polymeric nanoparticles) have preference over the non-degradable ones, because there are chances of accumulating for non biodegradable ones are more pronounced.

Figure 1.1: Drug delivery carriers

Micelles: formed by self - assembly of amphiphilic block co-polymer (5-50 nm) in aqueous solutions are of great interest for drug delivery application. They can be physically entrapped in the core of block copolymer micelles and transported at concentration that can exceed their intrinsic water solubility.

Net Liquid Crystals: Liquid Crystals combine the properties of both liquid and solid states. They can be made to form different geometries, with alternative polar and non-polar layers (i.e., a lamellar phase) where aqueous drug solutions can be included.

Liposomes: Liposomes are made up of phospholipids and cholesterol. These are in the form of vesicles which consist of hydrophilic core surrounded by hydro-phobic lipid bi-layer. Liposomes show versatile applications in pharmaceutical and cosmoceutical formulations. Nano-liposomes are also vesicular in shape and their particle size is in the range of nanometers. Different factors influence the properties of liposomes such as composition of liposomes, particle size, charge on surface and

formulation method. Nano-liposomes are mostly used as carrier for anti-bacterials, anti-virals, insulin, anti-neoplastics and plasmid DNA.

Dendrimers: Dendrimer is derived from a Greek word Dendron which means a tree. Dendrimers are polymeric molecules made up of multiple perfectly branched monomers .Different polymers such as poly-amido-amine (PAMAM), melamine, poly-L-glutamic acid, poly-ethylene-imine (PEI), poly propylene-imine (PPI), and polyethylene glycol, and chitin are used in formulation of dendrimer. These are extensively applied in magnetic resonance imaging and targeting cancerous cells.

1.6 NANOPARTICLES

Nanoparticles are defined as particulate dispersions or solid particles with a size in therange of 10-1000 nm. The drug dissolved, entrapped, encapsulated or attached to a nanoparticles matrix. Depending upon to the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. [13]

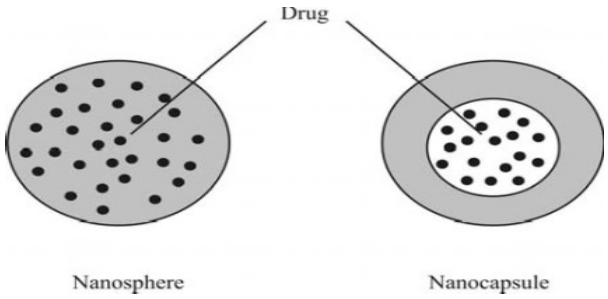


Figure 1.2: Difference between Nanosphere and Nanocapsule.

These polymeric materials are considered important due to their known biodegradability and biocompatibility. Several synthetic and natural origin polymers have been used for the preparation of Nanoparticles.

Targeted drug delivery can be achieved either by active targeting or passive targeting. Active targeting of drugs can be attained either by conjugating drug molecule with tissue specific or cell specific ligand. Whereas, passive targeting of drugs can be attained either by incorporating drug molecule into a nanoparticles.[14]

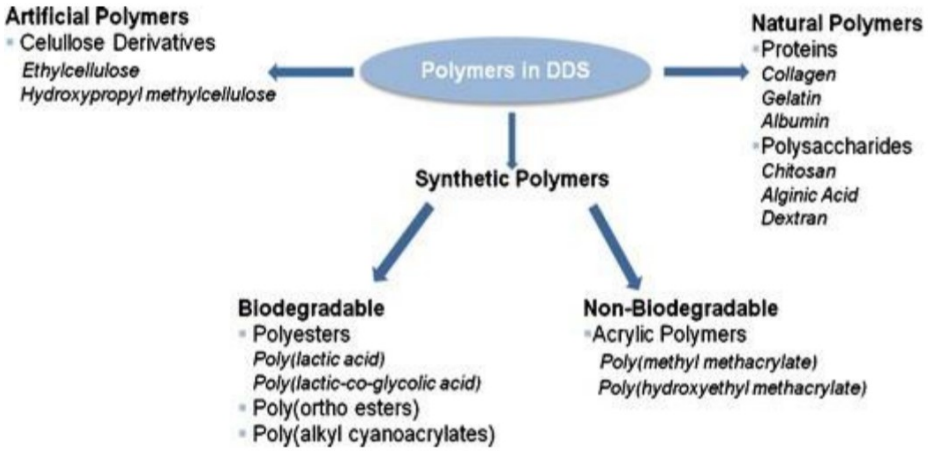


Figure 1.3: Polymers used in drug delivery system

Advantages of Nanoparticle over other novel drug delivery system[14]

- The allowable size of Nanoparticle to be administered *via* intravenously unlike colloidal system which could occlude in blood capillaries and needle
- Due to its small size than microspheres and liposomes, they can easily pass through the sinusoidal spaces in the bone marrow and spleen as compared to other systems with long circulation time.
- Due to their larger surface area, nanoparticles have higher loading capacity.
- It reduces the toxicity of liver.
- Nanoparticles increase stability of drug/proteins against enzymatic degradation.
- Nanoparticles are safe and effective in site specific and targeted drug delivery systems.
- To enhances the targeting moieties by adhering monoclonal antibodies with nanoparticles for specificity.
- It improves the solubility of poorly water soluble drugs.
- It improves bioavailability by reducing the fluctuations in the therapeutic ranges.
- They offer controlled rate of drug release and particle degradation characteristics that can be readily modulated by the choice of matrix constituents.

Disadvantages of nanoparticles[14]

- Difficult to manufacture in large scale.
- Due to their small particle size and large surface area can lead to particle-particle aggregation, making physical handling of NPs difficult in liquid and dry forms.
- Quickly scavenged by RES resulting in low biological half-life.
- Residual amount of organic solvent (nano- suspension) causes toxicity.
- Highly immunogenicity or foreignness.
- Long and expensive to cost.
- Chances of poor targeting.

Types of nanoparticles[15, 16]

Polymeric Nanoparticles: Biodegradable nanoparticles, as effective drug delivery system, are being applied extensively over a past few decades. Nanoparticles formulated from various natural and synthetic polymers have gained importance. This drug delivery system provides targeted drug delivery, increased bio-availability, and sustained release of drugs and protects drugs from enzymatic degradation.

Fullerenes: A fullerene is a molecule made up of carbon in different shapes such as tubes, hollow-sphere, and ellipsoid as mentioned. Fullerene is similar to graphite in structure.

Nanotubes: Nanotubes (NTs) are cylindrical fullerenes. NTs have a closed end as well as open end. Fullerenes show various therapeutic properties such as targeting cancerous cells, binding specific antibiotic to the specific structure of bacteria etc.

Solid lipid nanoparticles (SLNs): SLNs are lipids in nature which remain in solid phase at normal room temperature. SLNs are composed of solid hydrophobic core and a single coating layer of phospholipids. SLNs are stabilized by different surfactants for emulsification and also show many properties such as increased bio-degradability, increased bio-availability and drug targeting in the brain. SLNs have vast applications in cancer. SLNs have ability to accumulate tumor and also increase allow anticancer drugs delivery to the brain.

Super Paramagnetic Nanoparticles: These are attracted towards a specific magnetic field. When the magnetic field is removed, these cannot retain their residual magnetism. Particles range in the size of 5 nm to 100nm and used for selective

magnetic bio-separations and can be visualized in magnetic resonance imaging (MRI). These work on the principle of magnetic field and heated to trigger the drug release. These have also shown major role in cancer therapy and diagnosis.

Nanostructure lipid carriers (NLC): NLC are prepared by using blend of solid lipids and liquid lipids. The particles remain in solid state at normal room temperature. Nanostructure lipid carriers (NLC) and the lipid drug conjugate (LDC) nanoparticles are prepared in the form of matrices. These matrices increase drug loading capacity and bio-availability. These also have applications in the fields of cosmetics, food, agricultural and used in the delivery of anti-inflammatory drugs.

Nanoshells: Nanoshells also known as core-shells are spherical cores of concentric particles which are surrounded by an outer coating of thin layer of another material. Nanoshells have biomedical imaging and therapeutic applications.

Gold Nanorods: Gold nanorods were first time prepared in mid-1990. These exhibit distinct optical and electronic properties and depend on shape, size and aspect ratios. These can be easily stabilized, conjugated to antibodies.

Quantum Dots (QD): The QD are known as semiconductor nano-crystals and core-shell. These are 2 nm to 10 nm in size. These are used as drug delivery system for various hydrophilic drugs such as small interfering RNA and anti-sense oligo-deoxy-nucleotide as well as targeting antibodies, peptides etc. QD have extensive applications in imaging contrast.

Nanofibers: Nanofibers are produced by electro spinning technique in which fabrication of polymers in a fine and dense mesh works directly from solution and requires an electric field. These have dimension less than 100 nm as mentioned. Polymeric nanofibers are effective carriers for drug delivery and show advantages such as specific surface with small pore size, porosities, reduced toxicity and increased therapeutic level and bio-compatibility.

Ceramic nanoparticles: Ceramic nanoparticles are porous in nature and particle size is less than 50 nm. These possess distinct properties such as sol-gel process, work in ambient temperature condition and product produced of desired size, shape and porosity as well as effective in hiding the uptake by reticulo endothelial system.

Nanoerythrosomes: Nanoerythrosomes are derived from a red blood cell membrane by the process of haemo-dialysis through filter. Nanovesicles are of defined pore size and composed of proteins, phospholipids and cholesterol. These can load a variety of biologically active agents such proteins. Nanoerthroysomes composed of a natural membrane which allows the insertion of recombinant ligands along with better stability.

Method of preparation[17]

- Solvent Evaporation
- Solvent Displacement / Precipitation method
- Emulsification Diffusion
- Salting Out Dialysis
- Supercritical Fluid Technology (SCF)
- Polymerization in Emulsion
- Interfacial Polymerization
- Controlled/Living radical polymerization(C/LRP)
- Ionic Gelation or Coacervation Of Hydrophilic Polymers

Mechanism of drug release[18]

Controlled drug release and subsequent biodegradation are important for developing successful formulations.

Potential release mechanisms involve:

1. Desorption of surface-bound /adsorbed drugs
2. Diffusion through the carrier matrix
3. Diffusion (in the case of nanocapsules) through the carrier wall;
4. Carrier matrix erosion;
5. Combined erosion /diffusion process.

- Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time.
- Pulsatile release is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature).

1.7 NANOSPONGE DRUG DELIVERY SYSTEM

Introduction

The term "Nanosponge" means the nanoparticles having porous structures.[19] Nanosponges are tiny sponges with a size of about a virus with an average diameter below 1µm. Tiny sponges are incorporated in specific dosage form and circulate around the body until they encounter the specific target site and stick on the surface and began to release the drug in a controlled and predictable manner. Because the drug can be released at the specific target site instead of circulating throughout the body it will be more effective for a particular given dosage. Nanosponges are capable of providing solutions for several formulation related problems. Owing to their small size and porous nature they can bind poorly- soluble drugs within the matrix which leads to improve their solubility and the bioavailability of poorly soluble drugs. They can be crafted for targeting drugs to specific sites, prevent drug and protein degradation and prolong drug release in a controlled manner.[20]

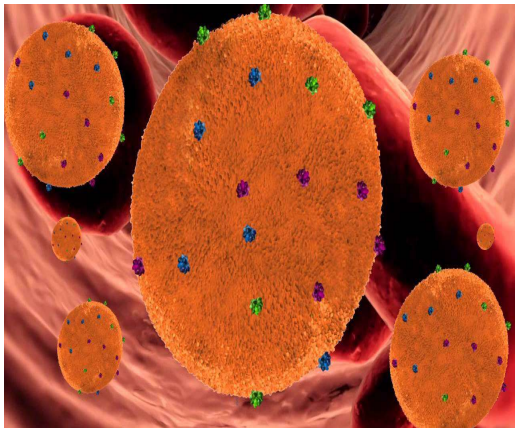


Fig.1.4: Polymer based nanosponges

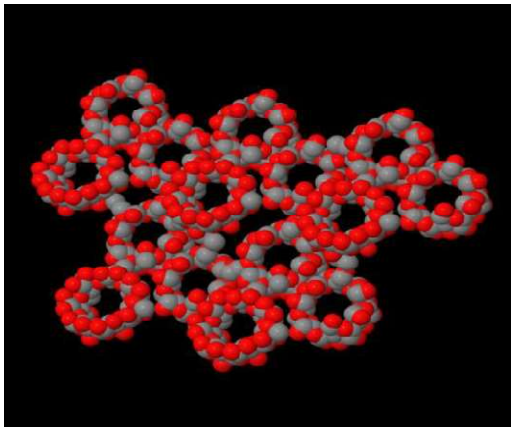


Fig.1.5: Cyclodextrin based nanosponges

Nanosponges are a novel class of nanoparticles with nanostructured hyper branched polymers and few nanometres wide cavities. Effective targeted drug delivery systems have been a dream for a long time, but it has been largely frustrated by the complex chemistry that is involved in the development of new systems. Targeting drug delivery has long been a problem for medical researchers i.e., how to get them to the right place in the body and how to control the release of the drug to prevent overdoses. Nanosponges are one of such effective drug delivery system which conquers this problem. The nanosponge drug delivery platform is a network of

specific polymers that slowly degrades and thus releases the chosen drug. Nanosponges are in nano size with sponge like morphology. Nanosponges were originally developed for topical delivery of drugs.[21]

Nanosponges are tiny mesh like structures in which large variety of substance can be encapsulated. That may revolutionize the treatment of many diseases and early trials suggest this technology is up to five times more effective at delivering drugs for breast cancer than conventional methods. They are able to capture, transport and selectively release a huge variety of substances because of their 3D structure containing cavities of nanometric size and tunable polarity. Furthermore, nanosponges show a remarkable advantage in comparison with the common nanoparticles: indeed, they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, stripping with moderately inert hot gasses, mild heating, or changing pH or ionic strength. For all these characteristics, nanosponges have been already employed in different applied fields, such as cosmetic and pharmaceutical sectors.[22]

They have a proven spherical colloidal nature, reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior.[23] Nanosponges can solubilize poorly water soluble drug and provide prolonged release as well as improving drugs bioavailability.[24] Nanasponges are able to load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility.[25] It is possible to control the size of nanosponges by varying the concentration of polymer to cross linkers.[26] Nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core. By the method of associating with drugs, the nanoparticles can be classified into:

- **Encapsulating nanoparticles**: These are represented by nanosponges and nanocapsules. Nanosponges containing many holes that carry the drug molecules. Nanocapsules such as poly (isobutyl-cyanoacrylate) are also encapsulating nanoparticles. They can entrap drug molecules in their aqueous core.

- **Complexing nanoparticles:** These nanoparticle attract the molecule by electrostatic charges.

- **Conjugating nanoparticles:** These nanoparticles links to drug through strong covalent bonds.[27]

The nanosponge is about the size of a virus with a 'backbone' (a scaffold structure) of naturally degradable polyester. The long length polyester strands are mixed in solution with small molecules called cross-linkers that have an affinity for certain portions of the polyester. They 'cross link' segments of the polyester to form a spherical shape that has many pockets (or cavities) where drugs can be stored. The polyester is predictably biodegradable, which means that when it breaks up in the body, the drug can be released on a known schedule.[28] The engineering capacity of nanosponge is due to the relatively simple chemistry of its polyesters and cross-linking peptides, compared to many other nano scale drug delivery systems. These nanosponges can be magnetized when they are prepared in the presence of compounds having magnetic properties.[29]

Nanosponges are water soluble but does not break up chemically in water. They mix with water and use as a transport fluid. They can be used to mask unpleasant flavors, to convert liquid substances to solids. The chemical linkers enable the nanosponges to bind preferentially to the target site. The nanosponges are solid in nature and can be formulated as Oral, Parenteral, Topical or Inhalation dosage forms. For the oral administration, the Complexes may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents suitable for the preparation of capsules or tablets. For topical administration they can be effectively incorporated into topical hydrogel. For parenteral administration, these can be simply mixed with sterile water, saline or other aqueous solutions.[30]

Characteristics of Nanosponges[31, 32, 33]

- Nanosponges provide a range of dimensions (1 μm or less) with tunable polarity of the cavities.
- Nanosponges are porous particles having high aqueous solubility, used mainly to encapsulate the poor soluble drugs.
- They can be reproduced by simple thermal desorption, extraction with solvents, by using microwaves and ultrasounds.
- These Nanosponges are capable of carrying both lipophilic and hydrophilic drugs.
- They protect the drug from physicochemical degradation.

- Nanosponges as formulations are stable over the pH range of 1 to 11 and temperature up to 130°C.
- Nanosponges are non irritating and non-mutagenic, non-allergic and nontoxic Biodegradable.
- The drug profiles can be varying from fast, medium to slow release in case of dosing therapy.
- Nanosponges can encapsulate various types of molecules by forming inclusion and non inclusion complexes.
- Chemical linkers permit nanosponges to bind preferably to the target site.
- Their three-dimensional structure allows capture, transportation and selective release of a variety of substances.

Advantages of Nanosonges[19, 22, 34]

- Targeted site specific drug delivery.
- They increase the solubility of poorly soluble drug.
- Less harmful side effects (since smaller quantities of the drug have contact with healthy tissue).
- They increase the bioavailability of drug.
- These formulations are compatible with most vehicles and ingredients.
- These formulations are free flowing and can be cost effective.
- This Technology offers entrapment of wide variety of ingredients and reduced side effects.
- Improved Stability, increased elegance and enhanced formulation flexibility.
- These are self-sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate.
- A nanosponge provides continuous action up to 12 hours i.e. extended release.
- It minimizes the irritation and it gives better tolerance which leads to improved patient compliance.
- Allows incorporation of immiscible liquids which improves material processing, liquid can be converted to powders.
- Can mask the unpleasant flavors and potential to convert liquid substance to solid substance
- Controllable and predictable release of drug.
- The preparation and development method requires simple chemistry.

- Particles can be made smaller or larger by varying the proportion of cross-linker to polymer.

Disadvantages of Nanosonges[19, 34]

- It depends upon loading capacities drug molecules.
- It includes only small molecules, not large molecules.

Composition and structure of Nanosponges[35, 36,3 7]

Nanosponges are complex structures, normally built up from long linear molecules that are folded by cross linking into a more or less spherical structure, about the size of a protein. Typical nanosponges have been constructed from cyclodextrin cross linked with organic carbonates. Nanosponges mainly consists three components. They are,

A. Polymer B. Cross linking agent C. Drug substance.

A. **Polymer:** Type of polymer used can influence the formation as well as the performance of Nanosponges. For complexation, the cavity size of nanosponge should be suitable to accommodate a drug molecule of particular size. The ability of the polymer to be cross-linked depends on the functional groups and active groups to be substituted. The selection of polymer depends on the required release and the drug to be enclosed. The polymers can be used to enclose the drug or to interact with the drug substance. For the targeted drug release the polymer should have the property to attach with the specific ligands.

B. **Crosslinking agent:** Selection of crosslinking agent depends on the structure of polymer and the drug to be formulated. The list of polymers and crosslinking agents used for the synthesis of nanosponges are presented in Table No.1.2.

C. **Drug substance:** Drug molecules to be formulated as nanosponges should have certain characteristics mentioned below.

- Molecular weight between 100 and 400 Daltons.
- Drug molecule consists of less than five condensed rings.
- Solubility in water is less than 10 mg/ml.
- Melting point of the substance is below 250^0C.

Table No.1.1: Chemicals used for synthesis of Nanosponges

Polymers	Hyper cross linked Polystyrenes, Cyclodextrins and its derivatives like Methyl β-Cyclodextrin, Alkyloxycarbonyl Cyclodextrins, 2-Hydroxy Propyl β-Cyclodextrins, Ethyl Cellulose, Polymethylmethacrylate.
Copolymers	Poly (valerolactone-allylvalerolactone oxepanedione) , Poly vinyl alcohol.
Crosslinkers	Dichloromethane, Carbonyl diimidazoles , Carboxylic acid dianhydrides, Diarylcarbonates, Diisocyanates , Epichloridine 2,2-bis(acrylamido) Acetic acid Diphenyl Carbonate, Pyromellitic anhydride,Gluteraldehyde.

Methods of preparation of Nanosponges

Nanosponges are prepared depending on type of delivery system. Nanosponges can be prepared by optimizing formulation parameters such as drug:polymer ratio, polymer:crosslinking agent ratio and agitation or stirring speed.[35]

Emulsion solvent diffusion method[35, 38]

In this method the two phases used are organic and aqueous. Aqueous phase consists of polyvinyl alcohol and organic phase include drug and polymer. After dissolving drug and polymer to suitable organic solvent, this phase is added slowly to the aqueous phase and stirred for two or more hours and then Nanosponges are collected by filtration, washed and then dried in air at room temperature or in vacuum oven 40^0C for 24 hrs.

Ultrasound-assisted synthesis[21, 39]

Nanosponges can be prepared by reacting polymers with cross-linkers in absence of solvent under sonication. In this method, the polymer is mixed with the cross-linker in a appropriate molar ratio in a flask. The flask is then placed in ultrasound bath which is filled by water and heated at 90°C. Sonication of the above mixture is done for few hours. Then, the above mixture is to be cooled and product obtained is broken roughly. The product is washed by water to remove non-reacted

polymer and purified by using soxhlet extraction using ethanol and further drying will give Nanosponges.

Solvent method[35, 40]

Mix the polymer with a suitable solvent, in particular polar aprotic solvent such as di methyl formamide, di methyl sulfoxide. Then add this mixture to excess quantity of the cross-linker, preferably in crosslinker/polymer molar ratio of 4 to 16. Carry out the reaction at temperature ranging from 10°C to the reflux temperature of the solvent, for time ranging from 1 to 48 hrs. Preferred crosslinkers are carbonyl compounds (Di methyl carbonate and Carbonyl di imidazole). After completion of the reaction, allow the solution to cool at room temperature, then add the product to large excess of bi distilled water and recover the product by filtration under vacuum and subsequently purify by prolonged soxhlet extraction with ethanol. Dry the product under vacuum and grind in a mechanical mill to obtain homogeneous powder.

Nanosponges prepared from Hyper Cross Linked β-Cyclodextrins[27, 29]

They are obtained by reacting cyclodextrin with a cross-linker such as di isocianates, diaryl carbonates, Dimethyl carbonate, diphenyl carbonate, and carbonyl di-imidazoles, carboxylic acid dianhydrides and 2, 2-Bis (acrylamido) acetic acid. The surface charge density, porosity and pore sizes of sponges can be controlled to attach different molecules. Nanosponge with low cross linking gives a fast drug release.

β-cyclodextrin nanosponges were prepared as 100ml of dimethyl Formaamide (DMF) was placed in a round bottomed flask and 17.42g of anhydrous β-CD was added to achieve complete dissolution. Then 9.96g of carbonyl di-imidazole (61.42m mol) was added and the solution allowed reacting for 4 hrs at 100^0c. Once condensation polymerization was complete, the transparent block of hyper cross linked cyclodextrin was roughly ground and an excess of deionised water added to remove DMF. Finally residual by-products or unreacted reagents were completely removed by soxhlet extraction with ethanol.The white powder thus obtained was dried overnight in an oven at 60^0c and in a mortar. The fine powder obtained was dispersed in water. The colloidal part that remained suspended in water was recovered and lyophilized. The obtained Nanosponges are sub-micron in dimension and with a spherical shape.

Loading of Drug IntoNanosponges[21, 35]

Nanosponges should be pre-treated to obtain an average particle size below 500nm. Nanosponges are suspended in water and sonicate to avoid the presence of aggregates and then centrifuge the suspension to obtained colloidal fraction. Supernatant is separated and sample is dried under freeze drying. Aqueous suspension of Nanosponges is prepared. The excess amount of drug is dispersed in aqueous suspension of Nanosponges and maintained the suspension under constant stirring for specific time required for complexation. After complexation, separate the uncomplexed drug from complex drug by centrifugation. Then obtained the solid crystals of Nanosponges by freeze drying or solvent evaporation. Crystal structure of Nanosponges plays a very important role in complex formation with drug. A study revealed that paracrystalline nanosponges showed different loading capacities when compared to crystalline nanosponges.

FACTORS INFLUENCING NANOSPONGES FORMATION [31, 35]

Type of polymer: Type of polymer used can influence the formation as well as the performance of nanosponges. For complexation, the cavity size of nanosponges should be suitable to accommodate a drug molecule of particular size.

Type of drug: Drug molecules to be complexed with nanosponges should have certain characteristics as mentioned above.

Temperature: Temperature changes can affect drug/nanosponges complexation. In general, increase in the temperature decrease the magnitude of the apparent stability constant of the drug/nanosponges complex which may be due to a result of possible reduction of drug/nanosponges interaction forces, such as van-der Waal forces and hydrophobic forces with rise of temperature.

Method of Preparation: The method of loading drug into the nanosponges can affect drug/nanosponge complexation. However, the effectiveness of a method depends on the nature of the drug and polymer, in many cases freeze drying was found to be most effective method for drug complexation.

Degree of Substitution: The complexation ability of the nanosponges may be greatly affected by type, number and position of the substituent on the parent molecule.

Higher the number of substituents, higher the crosslinking ability. Increase in the degree of crosslinking leads to formation of highly porous nanosponges due to more interconnections between polymers forming a mesh type network.

CHARACTERIZATION AND EVALUATION OF NANOSPONGES

Inclusion complexes formed between the drug and nanosponges can be characterized by following methods.

Thermo-analytical methods [19]

The most commonly used methods are DSC and DTA to observe the peak broadening, peak shifting and appearance and disappearance of certain peaks with the help of thermogram. These thermo-analytical methods determine whether the drug substance undergoes some change before the thermal degradation of the Nanosponge. This degradation may be melting, evaporation, decomposition, oxidation or polymorphic transition. The change of the drug substance indicates the complex formation.

Zeta potential [19]

Zeta sizer can be used to measure zeta potential, which is the measure of surface charge of Nansponges. Zeta potential is widely used for quantification of the magnitude of the electrical surface charge at the double layer. The significance of zeta potential is that its value can be related to the stability of formulation. More than 30 mV zeta potential value in water indicates good stability of Nanosponge.

Fourier Transform Infrared (FTIR) Analysis [27, 28]

Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer in solid state. Samples were scanned in the range from 400-4000 cm^{-1}

X-ray diffractiometry[19]

Powder X-ray diffractometry can be used to detect inclusion complexation in the solid state. The complex formation of drug with nanosponges alters the diffraction patterns and also changes the crystalline nature of the drug. When the drug molecule is liquid (since liquid have no diffraction pattern of their own), the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed nanosponge. This difference of diffraction pattern indicates the complex formation. The complex

formation of drug with nanosponges alters the diffraction patterns and also changes the crystalline nature of the drug. The complex formation leads to the sharpening of the existing peaks, appearance of a few new peaks and shifting of certain peaks.

Morphology and surface topography [22]

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) was used to analyze particle size, shape and surface morphology and surface topography of the drug.

Single Crystal X-Ray Structure Analysis[35]

The detailed inclusion structure and mode of interaction can be determined by Single Crystal X-Ray Structure Analysis. It can also help to determine the interactions between the host and guest molecules and the precise geometrical relationship can be established.

Production Yield [30]

The production yield (PY) can be determined by calculating initial weight of raw materials and final weight of nanosponges.

$$\text{Production Yield} = \frac{\text{Practical mass of Nanosponge}}{\text{Theoretical mass (polymer + drug)}} \times 100$$

Porosity [32]

Porosity study is carried out to check the Nanochannels and nanocavities formed in Nanosponge. Porosity of Nanosponge is determined by Helium displacement method using helium pynometer. Owing to their porous nature, nanosponges exhibit higher porosity compared to the parent polymer used to fabricate the system. Percent porosity is given by equation

$$\%\text{Porosity} = \text{Bulk volume} - \text{True volume} / \text{Bulk volume} \times 100$$

Loading Efficiency / Entrapment Efficiency [40]

The weighed amount of drug loaded NPs dispersed in suitable solvent and after sonication for specific period of time, sonication required to break the complexes. After dilution, it is analyzed by UV spectrophotometer or HPLC method.

$$\text{Loading efficiency} = \text{Actual drug content in Nanosponge} / \text{Theoretical drug content} \times 100$$

Particle size and Polydispersibility index (PDI) [19, 3 8]

The particle size and polydispersity index(index of width or spread or variation within the particle size distribution) can be determined by dynamic light scattering using 90 Plus particle sizer equipped with MAS OPTION particle sizing software or laser light diffractometry or Malvern Zeta sizer. From this, the mean diameter and polydispersity index can be determined.

Solubility Studies[29]

The Inclusion complexation are studied by phase solubility method described by Higuchi and Connors, which examines the effect of a nanosponge, on the solubility of drug. Phase solubility diagrams indicate the degree of complexation

***In vitro* release studies [37]**

The In-vitro release study can be carry out with optimize formulation of nanosponges by using multi-compartment rotating cell with dialysis membrane. USP II can be used in many cases depending upon the formulation.

Drug release kinetics [37]

To investigate the mechanism of drug release from Nanosponge. The release data was analysed using Zero order, First order, Higuchi, Korsemeyer-Peppas, Hixon Crowell.

Accelerated stability study[19]

Stability study carry out for Nanosponge formulation by placing the freshly prepared samples in stability chamber as per the ICH guidelines.

Thin Layer Chromatography[36]

In TLC, the Rf values of a drug molecule diminishes to considerable extent and this helps in identifying the complex formation between the drug and nanosponge.

Swelling and water uptake [32]

For swellable polymers like polyamidoamine nanosponges, water uptake can be determined by soaking the prepared nanosponges in aqueous solvent. Swelling and water uptake can be calculated using equations

% Swelling=Marking of cylinder at a specified time point/Initial marking before soaking×100

% Water uptake=Mass of hydrogel after 72 hrs/Initial mass of dry polymer×100

True Density [32]

True density of nanosponges can be determined using an ultra-pycnometer under helium gas

Resiliency (Viscoelastic properties)[37]

Resiliency of sponges can be modified to produce beadlets that is softer or firmer according to the needs of the final formulation. Increased crosslinking tends to slow down the rate of release.

MECHANISM OF DRUG RELEASE FROM NANOSPONGES[29]

The sponge particles have an open structure and the active is free to move in and out from the particles and into the vehicle until equilibrium is reached. In case of topical delivery, once the finished product is applied to the skin, the active that is already in the vehicle will be absorbed into the skin, depleting the vehicle, which will become unsaturated, therefore disturbing the equilibrium. This will start a flow of the active from the sponge particle into the vehicle and from it to the skin until the vehicle is either dried or absorbed. Even after that the sponge particles retained on the surface of stratum corneum will continue to gradually release the active to the skin, providing prolonged release over time.

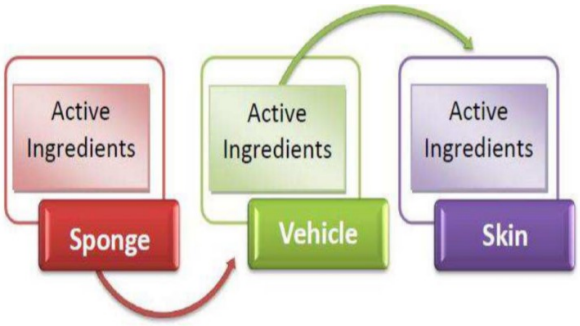


Figure 1.6: Drug release mechanism in topical delivery

Factors affecting drug release from Nanosponges[29]

- Physical and chemical properties of entrapped actives.
- Physical properties of sponge system like pore diameter, pore volume, resiliency.
- Properties of vehicle in which the sponges are finally dispersed.
- Particle size, pore characteristics, compositions can be considered as imperative parameters.

- External triggers like pressure, temperature and solubility of actives.
- Pressure: Pressure or rubbing can release drug from NPs onto skin.
- Temperature: Some entrapped actives can be too viscous at room temperature to flow spontaneously from sponges onto the skin but increased skin or environment temperature can result in increased flow rate and ultimately drug release.
- Solubility: Sponges loaded with water-soluble ingredients like antiperspirants and antiseptics release the ingredient in the presence of water.

APPLICATIONS OF NANOSPONGES

Cancer Therapy[19]

The anticancer drugs can be encapsulated with nanosponges. The nanosponge drug delivery system is three to five times more effective than direct injection. In that, the nanosponges are attach to the tumour cells or sucked by cells. The off-load their deadly content in controlled manner. Benefits of targeted drug delivery include more effective treatment at the same dose and fewer side effects. The drugs which are currently used as anticancer agents are paclitaxel, camptothecin etc.

Sustained delivery system [22]

The drug release kinetics from nanosponges can be obtained with a prolonged release profile over time by using suitable polymers and crosslinking agents. Nanosponges also can be used to store and prolong the release of volatile molecules, such as essential oils, following their encapsulation.

Protection from light or degradation[22]

Nanosponges can also be used as carriers to protect encapsulated molecules from light or chemical and enzyme induced degradation. Thus improving stability and retaining potency of the molecule.

Solubility enhancement[41]

The Nanosponge system has pores, which increase the rate of solubilisation of poorly soluble drug by entrapping such drugs in pores. Due to nanosize surface area significantly increased and increase rate of solubilisation. BCS class-2 drugs having low solubility and a dissolution rate limited poor bioavailability. However, when formulated with Nanosponge they demonstrate enhanced solubilisation efficiency,

with desired drug release characteristics. List of some BCS Class II dugs which can be developed as Nanosponges are given table 1.2.

Table No. 1.2: Biopharmaceutical classification system class II drugs.

Category of drug	List of drug
Antianxiety drugs	Lorazepam
Antiarrhythmic agents	Amiodarone hydrochloride
Antibiotics	Azithromycin, Ciprofloxacin, Erythromycin.
Anticoagulant	Warfarin
Anticonvulsants	Carbamazepine, Clonazepam, Oxycarbazepine,
Antidiabetic and Antihyperlipidemic drugs	Atorvastatin, Fenofibrate, Glibenclamide, Glipizi
Antiepileptic drugs	Phenytoin
Antifungal agents	Econazole nitrate, Griseofulvin, Itraconazole,
Antihistamines	Terfenadine
Antihypertensive drugs	Felodipine, Nicardipine, Nifedipine, Nisoldipine
Antineoplastic agents	Camptothecin, Docetaxel, Etoposide, Exemestane, Flutamide, Irinotecan,Paclitaxel, Raloxifene, Tamoxifen, Temozolamide, Topotecan
Antipsychotic drugs	Chlorpromazine Hydrochloride
Antiretrovirals	Indinavir, Nelfinavir, Ritonavir, Saquinavir
Antiulcer drugs	Lansoprazole, Omeprazole
Antioxidants	Resveratrol
Anthelmintics	Albendazole, Mebendazole, Praziquantel
Cardiac drugs	Carvedilol, Digoxin, Talinolol
Diuretics	Chlorthalidone, Spironolactone
Gastroprokinetic agent	Cisapride
NSAIDs	Dapsone, Diclofenac, Diflunisal, Etodolac.
Steroids	Danazol, Dexamethazone
Immunosupressants	Cyclosporine, Sirolimus, Tacrolimus

Antiviral application[28]

Nanosponges can be useful in the ocular, nasal, pulmonary administration routes. The selective delivery of antiviral drugs or small interfering RNA (siRNA) to the nasal epithelia & lungs can be accomplished by nanocarriers in order to target viruses that infect the RTI such as respiratory sinctial virus, influenza virus and rhinovirus. They can also be used for HIV, HBV, and HSV. The drugs which are currently in use as nanosponges delivery system are zidovudine, saquinavir, interferon- α, acyclovir (Eudragit based).

Blood Purification[19]

Blood purification can possible with the help of Nanosponges. Kidney failure is marked by accumulation of many middle Molecular weight toxins (MMW 10–20

has been done using haemodialysis. Dialysis Membranes allow permeation of low molecular weight solutes but the removal of potent MMW toxins remains incomplete. Hence, Malik et al. investigated a more specific technique for selectively allowing the MMW toxins to diffuse into the porous matrix while size-excluding serum albumins. They used a membrane emulsification technique.

Gas drug delivery system [19]

Various types of gases can be administered to the patient through the Nanosponges drug delivery system like oxygen, carbon dioxide, 1-methylcyclopropane etc. Nanosponges are able to store and release oxygen to the hypoxic tissue in various diseased state. The oxygen delivered to the hypoxic tissue by forming inclusion complexation with cyclodextrin based carbonate nanosponges. Generally, among the three types of cyclodextrins like α, β, γ-cyclodextrin, β-cyclodextrin is most commonly used, which previously saturated with oxygen before administration.

OtherApplications[29]

- Analytical Application,
- As Chemical Sensors
- Water Purification
- For Hydrogen storage
- In Agriculture,
- In Floriculture,
- In Food Industry
- For Oil Cleaning
- As Novel flame Retardants
- Against pore forming Toxins and superbug infections, and
- Micropatterning of Mammalian cell

Comparison of Some Effective Vesicular Systems[41]

Liposome, niosome, ethosome, transferosome and nanosponge are colloidal drug delivery systems. They all are nanometric in size. Liposome, noisome and transferosomes have some stability problems which is discuss below in table but Nanosponge enhanced the stability of drug.

Table No.1.3: Comparison of Nanosponge with Vesicular system

Liposome	Niosome	Ethosome	Transferosome	Nanosponge
Liposome consists of one or more concentric lipid bilayers, which enclose an internal aqueous volume.	Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids.	Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water.	Transferoso-mes are vesicular system consisting of phosphatidyl choline and surfactant.	Nanosponge are novel class of hyper-crosslinked polymer based colloidal structures consisting of solid nanoparticls with colloidal sizes and nanosized cavities.
The composition of liposomes is phospholipids and cholesterol.	They composed of non-ionic surfactants and cholesterol.	They composed mainly of phospholipids, high concentration of ethanol and water.	They consist of phospholipid and surfactants.	They composed of polymers and cross linkers.
Stability problems: due the formation of ice crystals in liposomes, the subsequent instability of bilayers leads to the leakage of entrapped material. The oxidation of cholesterol and phospholipids also leads to the formulation instability.	Stability problems: fusion, aggregation, Sedimentation and leakage on storage. The Hydrolysis of encapsulated drug.	Ethosomes has initiated a new area in vesicular research for transdermal drug delivery which can provide better skin permeation and stability than liposomes. Application of ethosomes provides the advantages such as improved entrapment and physical stability.	Stability problem: chemically unstable because of their pre disposition to oxidative degradation.	Nanosponge are chemically and physically stable. They increase the stability and bioavailabiliy modify drug release and reduce side effects.

Table No.1.4: Some examples of Nanosponges drug delivery with their formulations[41]

Drug	Nanosponges vehicle	Therapeutic activity	Attributes	Administ-ration route
Voriconazole	Ethyl cellulose, Poly (methyl methacrylate), Pluronic F-68	Antifungal	controlled release	Oral, topical
Atorvastatin	Ethyl cellulose, β-Cyclodextrin	Anti hyperlipidemic	Enhanced bioavailability	Oral
Econazole nitrate	Ethyl cellulose Polyvinyl alcohol	Antifungal	Enhanced drug solubility	Oral, topical
Isoniazid	Ethyl cellulose Polyvinyl alcohol	Ant tubercular	Enhanced drug solubility	Oral
Ciprofloxacin	Ethyl cellulose Polyvinyl alcohol	Antiulcer	Enhanced bioavailability	Oral
Flurbiprofen	β-CD, di phenyl carbonate	Anti-inflammatory	Sustained drug release	Oral
Doxorubicin	β-CD, di phenyl carbonate	Antineoplastic	Sustained drug release	Parenteral
Tamoxifen	β-CD, carbonyl diimidazole	Antiestrogen	Enhanced bioavailability, solubility	Oral
Paclitaxel	β-cyclodextrin	Antineoplastic	Enhanced bioavailability Cytotoxicty	Parenteral
Camptothecin	β-cyclodextrin	Antineoplastic	Haemolytic activity, Cytotoxicty	Parenteral
Itraconazole	β-CD,	Antifungal	Enhanced drug solubility	Oral, topical

Table No.1.5: Marketed preparation of Nanosponges[42]

Drug	Administration route	Trade name	Dosage form
Dexamethasone	Dermal	Glymesason	Tablet
Iodine	Topical	Mena- gargle	Solution
Alprostadil	I.V	Prostavastin	Injection
Piroxicam	Oral	Brexin	Capsule

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

LITERATURE SURVEY ON NANOSPONGE DELIVERY

1. **Pandey J *et al.*** [43] formulated and evaluated Nanosponge based controlled release topical gel preparation of Ketoconazole. Six batches of Nanosponges were prepared by solvent evaporation method using different proportion of EC and PVA. Nanosponges used for the preparation of gel using Carbopol 934 as gelling agent & penetration enhancer (Propylene glycol). Both the Nanosponges and gel were evaluated. Thereby they concluded that the Final batch (F6) is considered as a best entrapped and has a greater percentage drug release.

2. **Priyanka D *et* al.** [44] designed developed and evaluated Ibuprofen loaded Nanosponges for topical application. Nanosponges using different proportion of EC were prepared by solvent evaporation method using different proportion of EC. The Nanosponges were evaluated for FTIR, SEM, Physiochemical characteristics, *in vitro* dissolution study and drug entrapment efficiency. Thereby they concluded that the Nanospongs are suitable candidates for topical use and prolonged drug release.

3. **Dharshini MKP *et al*.** [45] developed and characterized an optimal stable Nanosponges formulation of Atorvastatin. Nine batches of Nanosponges were prepared using different ratios of PVA, beta cyclodextrin, Hp-β-Cyclodextrin and EC by solvent evaporation method to increase its bioavailability. Nanosponges were evaluated for drug content, entrapment efficiency, particle size, FTIR study, *in vitro* dissolution studies, kinetics analysis and stability studies. From the results of different evaluation parameters they concluded that amongst all the formulations AF8 shows high entrapment efficiency and complete drug release at the end of 12 hrs, also for highly lipophilic drug like Atorvastatin, Nanosponges approach would be possible alternative delivery system to conventional oral formulation to improve its bioavailability.

4. **Arvapally S *et al.*** [46] formulated and evaluated Glipizide Nanosponges. The nanosponges were prepared with different ratios of PVA, beta cyclodextrin, Hp-β-Cyclodextrin and EC by solvent evaporation technique and subsequently

formulated in a tablet form for sustained release of Glipizide. Prepared Nanosponges were evaluated in terms surface morphology, particle size, FTIR Study, production yield, and drug entrapment efficiency of Nanosponges. From the results of different evaluation parameters they concluded that amongst all the formulations the formulation GF4 has better results than other eight formulations. GF4 have its particle size 532.9nm, entrapment efficiency 94.40, drug content 98.6% drug release 99.71 % in 12 hrs.

5. **Shringirishni M *et al.*** [47] fabricated and characterized Nifedipine loaded β-Cyclodextrin Nanosponges. Nanosponges were prepared by Freeze drying method using different ratios of beta cyclodextrin and Di phenyl carbonate. Physiochemical characterization, drug entrapment efficiency, Surface characteristic by SEM, particle size, FTIR, DSC, stability studies, *in vitro* drug release, *in vivo* study and kinetic models of dissolution profiles were evaluated. Thereby they concluded that Nifidipine loaded cyclodextrin based Nanosponges potential drug delivery system for oral delivery of active molecule.

6. **Ansari A *et al.*** [48] prepared and evaluated Paclitaxel loaded Nanosponge to enhance oral Paclitaxel bioavailability. The prepared Nanosponge is evaluated for general test for Nanosponge and the pharmacokinetics of Paclitaxel through Nanosponge. The plasma concentration of paclitaxel after the oral administration was significantly higher than the Paclitaxel at the same dose shows the increased effect than conventional dose.

7. **Srinivas P *et al.*** [49] formulated and evaluated Isoniazid loaded Nanosponges for topical delivery. Nanosponges were prepared using by emulsion solvent evaporation method using ethyl cellulose as a polymer and PVA as surfactant. The effects of different surfactant concentration, drug: polymer ratio, stirring speeds, stirring time and sonication time on the physical characteristics of the Nanosponges as well as the drug entrapment efficiency, particle size analysis and surface morphology of the Nanosponges were investigated. From the above evaluation they concluded that the optimized Nanosponge formulation (I6) was selected for formulating nanogels using various gelling agents like Carbopol 934, Carbopol 940, and HPMC K4M and studied for pH, viscosity and *in vitro* drug

release. Thereby they concluded that the F2 was found to show the maximum sustained drug release of 74.26% in 10 hours.

8. **Aggarwal G *et al.*** [50] developed and compared the Nanosponge and Niosome based Gel for the topical delivery of Tazarotene**.** Nanosponge and Niosomes of Tazarotene were prepared by emulsion solvent evaporation technique and thin film hydration method respectively. The prepared formulations were characterized for drug content, morphology, size distribution, PDI, viscosity, % swelling and *in vitro* permeation. The optimized Nanosponge (F1) and Noisome (F5) formulations were incorporated into Carbomer 940 to convert them into Nanosponge and Niosome based gel. The gel formulations were subjected to drug content determination, pH determination, spreadability, viscosity, rheological behaviour and *in vitro* permeation studies using wistar rat skin by Franz diffusion cell for optimization. From the above evaluation they concluded that the FG1 in Nanosponge and FG5 in Niosome based gel showing promising result. So Nanosponge and Noisome formulations can be a possible alternative to conventional formulations of Tazarotene with enhanced bioavailability and skin retention characteristics for topical application.

9. **Penjuri SCB *et al.*** [51] formulated and evaluated Lansoprazole loaded Nanosponges. The Nanosponges were prepared by emulsion solvent diffusion method using ethyl cellulose, PVA and pluronic F-68.The prepared Nanosponges were evaluated for percentage yield, entrapment efficiency, particle size, FTIR study, Surface characteristic by SEM and *in vitro* drug release. From the above evaluation, the optimized F2 formulation was selected for formulated into enteric coated tablet and evaluated for weight variation, hardness, friability and dissolution studies. Thereby they concluded that the Nanosponges of Lansoprazole are promising for controlled drug delivery, which can reduce dosing frequency.

10. **Kumar PS *et al.*** [52] formulated and evaluated Miconazole nitrate loaded Nanosponges for vaginal drug delivery. Nanosponges were prepared using different ratios of beta cyclodextrin and Di phenyl carbonate. The Miconazole nitrate was loaded into the beta cyclodextrin. Nanosponges by solvent evaporation technique using various solvents. Nanosuspension was prepared with the

Miconazole Nanosponges. That nanosuspension is used for the preparation of Gel using Carbopol 934 as gelling agent. Both the Nanosponge and Gel were evaluated. Thereby they concluded that the F12 formulation has greater entrapment efficiency, good spreadability, extrudability, mucoadhesive nature and has sustained release.

11. **Jilsha G *et al.*** [53] formulated and evaluated Cephalaxin loaded hydrogel of Nanospong for topical delivery. Nanosponges were prepared by solvent diffusion method using hydroxy ethyl cellulose and PVA. High entrapment efficiency and least particle size of Nanosponges (F2) is used for the preparation of Hydrogel using Carbopol 934 as gelling agent with varying concentration of penetration enhancer (Proylene glycol). Both the Nanosponges and Hydrogel were evaluated. Thereby they conclude that the formulation having the higher concentration of permeation enhancer showed good skin permeation**.**

12. **Shankar G *et al.*** [54] formulated and evaluated β-cyclodextrin Nanosponges of poorly water soluble drug. β-cyclodextrin Nanosponge were prepared and Simavastatin was added to it. The Simvastatin Nanosponges were evaluated for the particle size, PDI, zeta potential, entrapment efficiency, FTIR, DSC, XRD, mucoadhesive strength, *in vitro* release study, kinetics study, and stability study. Thereby they concluded that the β-cyclodextrin based Nanosponge having higher solubilization and prolonged release of Simvastatin from Nanosponges. So the β-cyclodextrin based Nanosponge effective nanocarrier for the delivery of Simvastatin.

13. **Monica R *et al.*** [55] developed and evaluated Nanosponge based Pediatric controlled release dry suspension of Gabapentin for reconstitution. Nanosponges of Gabapentin were formulated using β-cyclodextrin by melt method. The Nanosponges drug complexes were characterized by FT-IR, DSC and PXRD as well as evaluated for taste and saturated solubility. Then it was coated with Ethyl cellulose and Eudragit RS 100. It shows the release of drug from Nanosponges was in controlled manner, as well as the taste of Gabapentin was masked. Thereby they conclude that the Nanosponge prepared with β-cyclodextrin was an ideal nanocarrier for controlled release.

14. **Seema G *et al.*** [56] developed and evaluated Curcumin loaded Nanosponges for colon drug delivery. Six batches of Nanosponges were prepared using Eudragit L-100 and zeta potential, drug content, drug entrapment efficiency, Surface characteristic by SEM, Particle size, FTIR, DSC, stability studies, *in vitro* drug release, and kinetic models of dissolution profiles were evaluated. Thereby they concluded that F4 formulation has found to be the optimum formulation, which has a significant improvement of Curcumin performance from Nanosponges compared to pure Curcumin and other formulation.

15. **Wang F *et al.*** [57] formulated hydrogel retaining toxin - absorbing Nanosponges for local treatment of Methicillin Resistant Staphlococcusaureus Infection. The Staphlococcusaureus which are resistant to the methicillin due to their toxin activity towards the drug. These types of toxins which were forming pore and constitute important bacterial virulence factors. These toxins disrupt cells by forming pores on cellular membranes and altering their permeability for bioactivity. Hybrid nanostructures like Nanosponges may has ability to absorb toxins and by reducing their effect. That can be proved by *in vitro* toxin Neutralization study, live whole body imaging of mice to study Nanosponges retention, *in vivo* toxin Neutralization study and *in vivo* detoxification efficacy against localized MRSA infection.

16. **Srinivas P *et al*.** [58] formulated and evaluated Voriconazole Nanosponges for oral and topical delivery as tablets and gel. Voriconazole Nanosponges were prepared by emulsion solvent evaporation technique with three different Polymers. Final formulations and Nanosponges were evaluated for drug content, entrapment efficiency, physical parameters, FTIR, *in vitro* and *in vivo* study and anti microbial activity. From the above evaluation they concluded that the three polymers used were efficient carriers for Voriconazole Nanosponges.

17. **Raja CH *et al.*** [59] fabricated and evaluated Ciprofloxacin loaded Nanosponges for sustained release. Five batches of Nanosponges using different proportion of Ethyl cellulose were prepared by solvent evaporation method. The Nanosponges were evaluated for Characterization by SEM, solubility study, *in vitro* dissolution study and drug entrapment efficiency. Thereby they concluded that the Final batch (F5) is considered as a best entrapped and has a greater percentage drug release.

18. **Rao M *et al.*** [60] fabricated and evaluated β-cyclodextrin-based nanosponges of Telmisartan to enhance the solubility and bioavailability. Nanosponges were formed by solvent evaporation method using cross-linking β-Cyclodextrin with carbonate bonds, NSs complexes of Telmisartan were characterized by DSC, XPRD, FTIR study, NMR, SEM, HPTLC analysis, Particle size analysis, zeta potential, porosity, *in vitro* dissolution studies, *in vivo* studies and solubility. Thereby they concluded that the highest solubility and *in vitro* drug release was observed in inclusion complex prepared from NS compared with plain Telmisartan.

19. **Sharma R *et al.*** [61] fabricated and evaluated Econazole nitrate Nanosponges as topical Hydrogel. Econazole Nanosponge were prepared by solvent evaporation technique using various concentration of ethyl cellulose. Nanosponges used for the preparation of Gel using Carbopol 934 as gelling agent & penetration enhancer (Proylene glycol). The Nanosponges were evaluated for entrapment efficiency, *in vitro* drug release, particle size, rheological properties. For hydrogel the Equilibrium swelling study, viscosity analysis, texture analysis, *in vitro* permeation study has evaluated. Thereby they concluded that the Nanosponges are suitable candidates for topical use & prolonged drug release.

20. **Swaminathan S *et al.*** [62] formulated and characterized Cyclodextrin-based Nanosponges encapsulating Camptothecin. Formulating complexes Camptothecin using three types of β-cyclodextrin with different ratio (1:2, 1:4 and 1:8). The prepared Nanosponges were evaluated for particle size, PDI, zeta potential, entrapment efficiency, FTIR, DSC, XRD, *in vitro* release study, kinetics study and stability study, cytotoxicity studies. Thereby they concluded that the β-cyclodextrin based Nanosponge show slow and prolonged Camptothecin release over a period of 24 hrs. The cytotoxicity studies on HT-29 cells showed that the Camptothecin formulations were more cytotoxic than plain Camptothecin after 24 hrs of incubation.

21. **Indira B *et al.*** [63] reviewed Nanosponges, a new era in drug delivery. They reviewed on the Nanosponges about the advantages, disadvantages, method of preparation, polymers and cross linkers used, factors influencing Nanosonges, characterization of Nanosponges and their applications. They concluded that this

new invention, Nanosponges will pave a way in overcoming the challenges in designing of targeted drug delivery systems because of their ability to accommodate either hydrophilic or lipophilic drugs and release them in a controlled and predictable manner at specific site in the body. The release rate can be modulated by controlling the polymer and cross linker ratio. The Nanosponges have been found to possess a profound ability to protect essential biomarkers in diseases (cancer for example) and biocatalysts from physicochemical degradation.

22. **Patel EK *et al.*** [24] reviewed Nanosponge and Microsponges a novel drug delivery system. They reviewed about all the aspect of Nanosponge like prepareation, evaluation, application, advantages, disadvantages, polymers and crosslinkers Used. Cyclodextrin based nanosponges are a novel class of cross-linked derivatives of cyclodextrins. They have been used to increase the solubility of poorly soluble actives, to protect the labile groups and control the release. They concluded that the Nanosponges are a Nanosized particle entraps wide variety of products, enhance solubility, provide prolonged use and provide patient compliance with the formulation.

LITERATURE REVIEW ON ANALYSIS OF LOVASTATIN

23. **Shraddha MG *et al.*** [64] formulated and evaluated the Lovastatin loaded Proniosomal powder, it can be converted into Niosomes immediately before use by hydration. Five batches Lovastatin Proniosomal powder prepared by slurry method using molar ratios of nonionic surfactants Span 60 with Cholesterol as membrane stabilizing agent and Maltodextrin a water soluble carrier. The proniosome formulations were evaluated for FT-IR study, angle of repose and SEM. The Niosomal suspensions were further evaluated for entrapment efficiency, *in vitro* release study, kinetic data analysis, stability study. From the above evaluation they concluded that the F3 formulation has higher entrapment efficiency (72.69%) and *in vitro* release was found to be 91.17 % at the end of 24hrs, the stability study reveals that the Proniosome formulations are stable when stored at 4°C.

24. **Priya K *et al.*** [65] developed and evaluated Lovastatin by Self-nano emulsifying drug delivery system (SNEDDS) using Acrysol EL 135 as oil phase, Lauro glycol

90 and Capmul MCM as surfactant and co-surfactant respectively to increase the solubility and bioavailability. SNEDDS formulations were evaluated for particle size, zeta potential, drug content, *in vitro* dissolution studies, SEM, stability studies, *in vivo* study, Pharmacokinetic analysis. From the above evaluation they concluded that the Formulation F8 was found to be best formulation among the all 14 formulation. Furthermore, pharmacokinetic studies in rats indicated, the optimized SNEDDS formulation significantly improved the oral bioavailability of Lovastatin compared to pure LOV.

25. **Suparna S *et al.*** [66] developed and evaluated liquid and solid Self-micro emulsifying drug delivery system of Lovastatin (SMEDDS). Using Labrafil M 1944, Acrysol EL 135 and Lauroglycol as oil, surfactant and co-surfactant respectively. The prepared systems were characterized for self-emulsification time, robustness to dilution, % transmittance, globule size and thermodynamic stability, DSC, X-ray powder diffraction studies, SEM studies, *in vitro* dissolution studies, *in vitro* absorption profile. Ternary phase diagrams were plotted to identify the area of micro emulsification. Thereby they concluded that the formulation F8 is considered enhanced *in vitro* dissolution and absorption profile.

26. **Patel S *et al.*** [67] developed and characterized Lovastatin loaded polymeric Nanoparticles for the treatment of hyperlipidemia. Nanoparticles (NPs) were prepared Using PMMA by solvent evaporation technique. The nanoparticles were characterized by SEM, particle size, zeta potential, entrapment efficiency, FTIR, DCM and x-ray diffraction analyses; also the *in vitro* drug release and *in vivo* hyperlipidemia activity are conducted in wistar rats. Thereby they concluded that the nanoparticles were capable of releasing the Lovastatin for prolonged period to maintain the constant plasma drug concentration.

27. **Fatima H *et al.*** [67] *f*ormulated and evaluated fast dissolving Lovastatin tablets solid dispersion using Eudrgit RS100 were successfully prepared by direct compression method. They were evaluated angle of repose, compressibility index, thickness, hardness, weight variation, friability. Thereby they concluded that the F4 formulation showed 100% drug release within 20 mins whereas marketed formulation showed 100% drug release in 60 mins.

28. **Guan Q *et al.*** [69] developed and evaluated Lovastatin loaded poly (lactic acid) microspheres for sustained oral delivery. Microspheres were prepared by improved emulsion-solvent evaporation method. Morphological examination, particle size, encapsulation ratio, drug loading, *in vitro* release and Pharmacokinetics studies were evaluated. Thereby they concluded that the poly (lactic acid) microspheres can significantly prolong the drug circulation time *in vivo* and can also significantly increase the relative bioavailability of the drug.

29. **Vinodh S *et al.*** [70] formulated and comparative studies of Lovastatin loaded polymeric Nanoparticles Prepared by ionic gelation and solvent evaporation technique using Sodium tripolyphosphate and Pleuronic F68 as surfactants to improve the solubility and dissolution characteristics of a poorly water soluble drug LOV. The prepared Nanoparticles were evaluated in terms of size, drug polymer compatibility by DSC, PDI, zeta potential, SEM, drug entrapment efficiency *in vitro* release and stability studies. Together, these results indicated that Nanoparticulate formulations are ideal carriers for oral administration of LOV having a great potential to improve the oral bioavailability and sustain the drug release, thereby minimizing the dose dependent adverse effects and maximizing the patient compliance.

30. **Bommakanti S *et al.*** [71] developed and evaluated Lovastatin tablets using floating drug delivery system. GRDDS of Lovastatin were prepared by wet granulation method using HPMC of different viscosity grades and Carbopol 934P and Chitosan each with different drug to polymer ratios. The prepared GRDDS were evaluated for various parameters. Thereby they concluded that the GRDDS of Lovastatin provides a better option for increasing the bioavailability and treating hypercholesterolemia.

31. **Vimal V *et al.*** [72] formulated, optimized and evaluated buccoadhesive delivery system of Lovastatin. buccoadhesive films were prepared using HPMC, Carbopol 934P and Poly vinyl alcohol. The patches were evaluated for their thickness, folding endurance, and weight uniformity, content uniformity, swelling behavior, mucoadhesive strength, surface pH, *in vitro* release studies, *ex vivo* buccal permeation study and accelerated stability studies. Thereby they concluded that

the buccal films of Lovastatin improves bioavailability and can be used as a potential drug delivery system in treatment of hypercholesterolemia.

32. **Seenivasan A *et al.*** [73] developed of a novel method for the quantification of Lovastatin. The estimation of Lovastatin produced by *Monascus purpureus* and pure Lovastatin was attempted by UV-visible spectrophotometer as well as HPLC. Thereby they concluded Pure Lovastatin can be quantified spectrophotometrically faster and cheaper than with HPLC having a high degree of accuracy.

33. **Viswanath V *et al.*** [74] enhanced the stability of Lovastatin by Liquisolid compaction technique using non volatile solvent like PEG 400 to produce the drug solution. This is mixed with the carrier material like Microcrystallin cellulose and the coating materials Aerosil, Sodium Starch Glycollateas super disintegrant, additives like magnesium stearate and lactose in a rapid mixer granulator and then sieved and dried and compacted. The formulated Lovastatin tablets were evaluated in terms of weight variation, hardness, friability test, disintegration test, uniformity of drug content, thickness and *in vitro* dissolution study. Thereby they concluded that the liquisolid compact technique can be used for enhancing the dissolution rate of Lovastatin tablets.

34. **Goyal U *et al.*** [75] formulated and evaluated Self-micro emulsifying drug delivery system of Lovastatin to overcoming the problems of poor solubility and bioavailability using Capryol 90 (20 %) as oil, Cremophore RH40 (40 %) as surfactant and Transcutol P (40 %) as co-surfactant. The prepared SMEDDS was characterized through its droplet size, zeta potential, emulsification time, rheological determination and transmission electron microscopy, *in vivo* pharmacodynamic studies using the Triton-induced hyperlipidemia model in Wistar rats. From pharmacodynamic studies, we concluded that the developed SMEDDS formulation of LOV shows superior lipid lowering activity compared to pure LOV. Thus our studies exemplified the promising use of SMEDDS to dispense lipid-soluble drugs by oral route.

35. **Basavaraj K *et al.*** [76] designed and characterized Nanocrystals of Lovastatin for solubility and dissolution enhancement. Nanocrystal of Lovastatin was formulated by simple precipitation method using acetone and methanol as a solvent without

using surfactant. The prepared Nanocystal were evaluated for particle size, SEM analysis, Crystalline state evaluation, powder X-ray diffraction (PXRD), DSC, solubility determination, *in vitro* release study, *in vivo* evaluation, dose for animal study, drug plasma study, stability study. Result shown that, the drug has enhanced saturation solubility, increased dissolution rate and more bioavailable in biological fluid when drug formulated by using acetone and methanol as a solvent. From the above evaluation they concluded that the acetone and methanol are the suitable solvents for the preparation of Lovastatin Nanocrystal.

36. **Vidyadhara S *et al.*** [77] formulated and evaluated Lovastatin fast dissolving tablets using newer super disintegrants. Polyethylene glycol-6000 as a carrier for solid dispersion techniques. The dispersions were prepared by employing different techniques such as physical mixing, fusion method and co-grinding methods. The prepared solid dispersions were compressed into tablets by direct compression along with solubility enhancing excipients like novel super-disintegrates such as Croscarmellose sodium, Crospovidone, Pregelatinized starch. These results indicated these solid dispersions exhibit faster dissolution characteristics when compared to pure drug. A higher dissolution rate was obtained with solid dispersions prepared by fusion method in the ratio of 1:2 for the drug and polymer. Thereby they concluded that Lovastatin tablets prepared by solid dispersions with Crospovidone as a super disintegrant was found to be ideal for rapid disintegration and for improving the dissolution rate which in turn increases the bioavailability.

37. **Anilkumar *et al.*** [78] developed and characterized biodegradable Chitosan Nanoparticles loaded with Lovastatin. Nanoparticles were prepared by modified ionotropic gelation method using factorial design. From the preliminary trials, the constraints for independent variables X1 (concentration of chitosan) and X2 (concentration of sodium tripolyphosphate) have been fixed and examined to investigate effect on particle size, encapsulation efficiency, zeta potential, *in vitro* drug release, SEM, FTIR, XRD and DSC analysis of Lovastatin. Thereby they concluded that the Lovastatin Nanoparticles could be effective in sustaining drug release for a prolonged period.

38. **Seenivasan A *et al.*** [79] formulated and characterized Lipid-based carriers such as solid lipid nanoparticle (SLNs), nano-structured lipid carriers (NLCs), and lipid emulsions (LEs) for better drug delivery of Lovastatin. Nanoparticles prepared by hot homogenization followed by ultrasonication method. The prepared nanoparticles were evaluated in terms of size, drug polymer compatibility by DSC, PDI, zeta potential, SEM, drug entrapment efficiency, *in vitro* release and stability studies. Thereby they concluded nano-aided drug delivery system is an appropriate option for poorly soluble lipophilic drugs.

39. **Shaikh K *et al.*** [80] formulated and evaluated poorly water Soluble drug of Lovastatin by solid dispersions techniques to improve its solubility and dissolution characteristics, reduce dosing frequency and to improve its stability. The prepared solid dispersions were characterized by FT-IR spectroscopy and evaluated for various parameters like drug content, solubility and dissolution studies and different physical properties. Result shown that, the dissolution data of all solid dispersions were increased compared to pure drug. From the above evaluation they concluded that the solvent evaporation method was found to be a promising method for formulating uniform and stable Lovastatin solid dispersions with enhanced surface area and dissolution rate.

40. **Suresh G *et al.*** [81] developed and characterized Lovastatin SLNs by hothomogenization followed by ultrasonication using lipids (trimyrstin and tripalmitin) with poloxamer 188 as surfactant zeta potential, drug content, drug entrapment efficiency, Surface characteristic by SEM, Particle size, FTIR, DSC, stability studies, *in vitro* drug release, and kinetic models of dissolution profiles were evaluated, also the bioavailability studies are conducted in male wistar rats. Thereby they concluded that the bioavailabilities of Lovastatin solid lipid nanoparticles were increased compared with reference Lovastatin suspension.

41. **Curran MP *et al.*** [82] compared the extended release Lovastatin with that immediate release at the same dose by randomized 4 week study in 24 patients. Result ER formulation provides smooth and sustained delivery, reducing LDL-C in patient significantly greater & adverse event were usually mild to moderate, the beneficial changes in lipid level are maintained during long term. Thereby they concluded that ER formulation greater efficacy then IR formulation.

AIM

&

PLAN OF WORK

3. AIM AND PLAN OF WORK

AIM OF WORK

- To formulate Lovastatin loaded Nanosponge using different polymer (Eudragit RS 100 polymer and Ethyl cellulose) by emulsion solvent evaporation method and characterize their properties.
- The current work is to enhance the rate of solubility of the drug, to augment bioavailability, minimize the dose dependent adverse effect and to release the drug in a controlled manner to improve the efficacy and patient compliance.
- To enclose Lovastatin Nanosponges in hard gelatin capsule.

OBJECTIVE OF WORK

- To improve the aqueous solubility and dissolution characteristics of a poorly water soluble drug of Lovastatin using nanotechnology.
- To improve the bioavailability & therapeutic efficacy of Lovastatin.
- To release the drug in a controlled manner for extended period of time..
- To improve the patient compliance and acceptance.
- To reduce the dose dependent side effects by the reduction of the dose (since smaller quantities of the drug have contact with healthy tissue).

PLAN OF WORK

The present work was planned to carry out the formulation and evaluation of Nanosponge loaded with Lovastatin

The design of work:

I. PREFORMULATION STUDIES:

1. Compatibility studies
   - Physical compatibility study
   - Chemical compatibility study by Fourier Transform Infra-Red Spectroscopy
2. Determination of melting point of pure drug and excipients
3. Determination of lambda max
4. Preparation of standard graph for Lovastatin
5. Solubility Studies of pure Lovastatin

II. FORMULATION OF LOVASTATIN LOADED NANOSPONGES

Formulation of Lovastatin loaded Nanosponges using different polymer (Ethyl cellulose and Eudragit RS 100) at different ratios (drug: polymer - 1:1, 1:2, 1:3, 1:4, and 1:5) with stabilizer (Polyvinyl alcohol) using Emulsion Solvent Evaporation Method.

III. EVALUATION OF PREPARED LOVASTATIN LOADED NPs

1. Determination of Production yield
2. Determination of Entrapment efficiency
3. Solubility studies of Lovastatin loaded Nanosponges
4. *In vitro* drug release studies
5. Morphology of Nanosponge by scanning electron microscopy (SEM) analysis
6. Determination of Particle size & Polydispersity by Malvern particle size analyzer
7. Determination of Zeta potential by Zeta sizer

IV. PREFORMULATION STUDY OF OPTIMIZED NANOSPONGES

Flow property of prepared Nanosponges are measured by

- Bulk density
- Tapped density
- Angle of repose
- Carr's index
- Hausner's ratio

Porosity

V. OPTIMIZED LOVASTATIN LOADED NANOSPONGES FILLED IN HARD GELATIN CAPSULES

VI. EVALUATION OF CAPSULES

- Uniformity of weight
- Drug Content
- Disintegration test
- *In vitro* release study

VII. RELEASE KINETICS OF OPTIMIZED FORMULATIONS

VIII. STABILITY STUDIES

RATIONALE OF THE STUDY

4. RATIONALE OF THE STUDY

RATIONALE OF THE DISEASE[83, 84]

- Hyperlipidemia is elevation of plasma cholesterol, triglycerides (TGs), or both, elevation of low density lipoprotein (LDL), or lowering of high-density lipoprotein (HDL) that contributes to the development of atherosclerosis. Atherosclerosis of the arterial vessel walls is the most important underlying cause of Cardio Vascular Diseases (CVD) and hyperlipidemia is a major and primary risk factor of atherosclerotic CVD.

- Cardiovascular diseases are the leading cause of death in the world, leading to almost 32% of all deaths in women and 27% in men in 2004. 35 million deaths (60% of total global mortality) annually are due to cardiovascular diseases.

- Statins- Atorvastatin, Simvastatin, Pravastatin and Lovastatin have been found to reduce deaths from heart attack among patients with history of heart disease or the risk factor for heart disease, such as diabetes and high blood pressure.

RATIONALE OF THE DRUG[85, 86]

- Lovastatin (LOV) is used to lower blood cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with Cardio Vascular Diseases (CVD) and statins are therefore used in the prevention of these diseases.
- After oral administration, Lovastatin is rapidly absorbed from the gastrointestinal tract and rapidly eliminated from plasma with a half-life of approximately 1-4 hrs.
- Lovastatin has low bioavailability (5%). Its belongs to BCS Class II (low solubility and high permeability), thus selected to augment the bioavailability of drug.
- Lovastatin is characterized by rapid clearance due to its shorter half life and thus warrants the use of sustained release formulation for prolonged action to improve its patient compliance.

RATIONALE OF DRUG DELIVERY SYSTEM[21, 22]

The reasons to select Nanosponge drug delivery are as follows:

- Nanosponges is a nano sized particle, has larger surface area, thereby the penetration is high when compared to other conventional products.
- Nanasponges are able to load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility.
- Low solubility drugs formulated as Nanosponge may increase the solubility and the bioavailability of poorly soluble drugs due to their small size and porous nature.
- It offers extended release upto 12 hours. This provides an extensive surface area for high entrapment and absorption of actives onto the polymeric cage, the release is prolonged.
- The engineering capacity of Nanosponge is due to the relatively simple chemistry of its polyesters and cross linkers, compared to many other Nano scale drug delivery systems
- It is possible to control the size of nanosponges by varying the concentration of polymer to cross linkers.
- It is stable over the wide range of pH 1 to 11 & temperature up to 130^0C. It also gives a high load but still it is free flowing.
- It is believed to contribute towards reduced side effect, Improved Stability, increased elegance and enhanced formulation flexibility.
- Nanosponges are non-irritating, non-mutagenic, nontoxic, non-allergenic and biodegradable.
- They protect the drug from physicochemical degradation.
- It can mask the unpleasant flavors and potential to convert liquid substance to solid substance.
- Chemical linkers permit nanosponges to bind preferably to the target site.
- Stability of Nanosponge is higher than most of the novel formulations.
- One of the best features of nanosponge is its self-sterilizing as their average pore size is 0.25μm where bacteria cannot penetrate into tunnel structure of Nanosponges.

DISEASE PROFILE

5. DISEASE PROFILE

HYPERLIPIDEMIA

Hyperlipidemia is a condition when abnormally high levels of lipids i.e. the fatty substances are found in the blood. This condition is also called hypercholesterolemia or hyperlipoproteinemia. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis related conditions like coronary heart disease (CHD), ischemic cerebrovascular disease, peripheral vascular disease and pancreatitis. The increase in lipids like low density lipoproteins (LDL), cholesterol (esters derivatives) and triglycerides are mainly responsible for this condition. These lipids are associated with blood plasma proteins and remain in the dissolved state in the blood.[87]

Lipids including fatty acids, cholesterol, phospholipids and other are found from exogenous sources (e.g. diet) or synthesized endogenously in the liver and intestines. Lipids have to be transported to the various tissues to accomplish their metabolic functions. Because of their insolubility, they are transported in the plasma in macromolecule complexes with proteins called lipoproteins.
The categories of lipoproteins include

- Chylomicrons (Cm)
- Very low density lipoprotein cholesterol (VLDL)
- Intermediate density lipoprotein cholesterol (ILDL)
- Low density lipoprotein cholesterol (LDL)
- High-density lipoprotein cholesterol (HDL)
- Lipoprotein(a) (Lp(a))

HDL is good because it carries extra cholesterol back to the liver where it can be eliminated. LDL is bad because it enables excess cholesterol to build up in the blood. Triglycerides are a type of fat in the blood. These are different from cholesterol, but because of their strong association with heart disease, triglycerides are also measured. A person with Hyperlipidemia may have high levels of both LDL and triglycerides.

In the fasting state, most plasma triglycerides are present in VLDL. In the non-fasting state, chylomicrons appear transiently and contribute significantly to total plasma triglyceride level. LDL carries about 70% of total plasma cholesterol but very

little triglycerides. HDL contains about 0% to 30% of plasma cholesterol. Generally, Hyperlipidemia is defined by serum total cholesterol >240mg/dL and /or LDL cholesterol > 160mg/dL and /or total cholesterol: HDL ratio >5.7 and/or total triglycerides ≥150mg/dL in adults.[88]

Description of Hyperlipidemia[89]

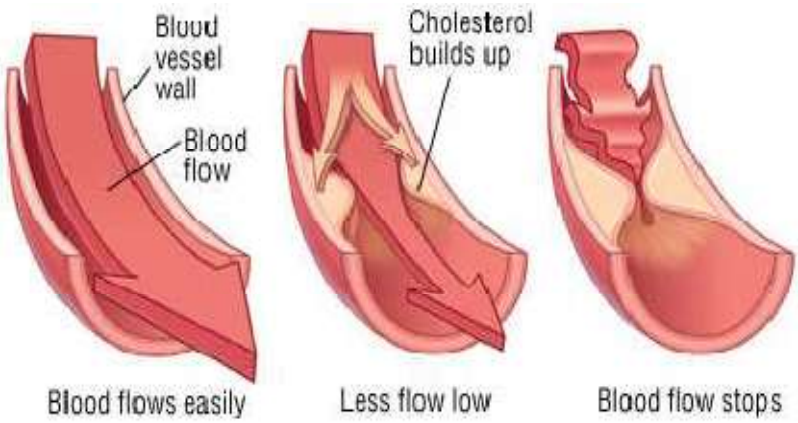


Figure 5.1: Hypercholesterolemia of blood vessel

- The fat-protein complexes in the blood are called lipoproteins. The best-known lipoproteins are LDL and HDL. Excess LDL cholesterol contributes to the blockage of arteries, which eventually leads to heart attack.
- Population studies have clearly shown that the higher the level of LDL cholesterol, the greater the risk of heart disease. In contrast the lower the level of HDL cholesterol, the greater the risk of coronary heart disease. As a result, HDL cholesterol is commonly referred to as the good cholesterol.
- Low HDL cholesterol levels are typically accompanied by an increase in blood triglyceride levels.
- Studies have shown that high triglyceride levels are associated with an increased risk of coronary heart disease. Although hyperlipidemia does not cause to feel bad, it can significantly increase the risk of developing coronary heart disease, also called coronary artery disease or coronary disease.
- People with coronary disease develop thickened or hardened arteries in the heart muscle. This can cause chest pain, a heart attack, or both. Because of these risks, treatment is often recommended for people with hyperlipidemia.
- High lipid levels can speed up a process called atherosclerosis, or hardening of the arteries. Arteries are normally smooth and unobstructed on the inside, but as age

goes, a sticky substance called plaque forms in the walls of your arteries. Plaque is made of lipids and other materials circulating in your blood. As more plaque builds up, your arteries can narrow and stiffen. Eventually, enough plaque may build up to reduce blood flow through your arteries.

- Hyperlipidemia has been implicated in atherosclerosis, which is the primary cause of heart disease and stroke. Atherosclerosis increases your risk of heart disease, stroke, and other vascular diseases. Fortunately, may be able to reduce high lipid levels and therefore prevent or slow the progression of atherosclerosis. Lifestyle changes like exercising and eating a healthy diet can also lower your lipid levels and are often the first step in treatment.

Epidemiology [90]

- In 2016, approximately 660,000 U.S. residents will have a new coronary event (defined as a first hospitalized myocardial infarction [MI] or atherosclerotic cardiovascular disease [ASCVD]) and approximately 305,000 will have a recurrent event.
- The estimated annual incidence of MI is 550,000 new and 200,000 recurrent attacks.
- The average age at first MI is 65.1 years for men and 72.0 years for women.
- Dyslipidemia is a primary, major risk factor for ASCVD and may even be a prerequisite for ASCVD, occurring before other major risk factors come into play.
- Epidemiologic data also suggest that hypercholesterolemia and perhaps coronary atherosclerosis itself are risk factors for ischemic cerebrovascular accident (CVA).
- According to data from 2009 to 2012, >100 million U.S. adults ≥20 years of age have total cholesterol levels ≥200 mg/dL; almost 31 million have levels ≥240 mg/ dL.
- Increasing evidence also points to insulin resistance - which results in increased levels of plasma triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) and a decreased concentration of high-density lipoprotein cholesterol (HDL-C) - as an important risk factor for peripheral vascular disease, CVA, and ASCVD.

Pathophysiology of Hyperlipidemia[89]

- Decreased clearance of triglyceride-rich lipoproteins due to inhibition of lipoprotein lipase and triglyceride lipase.

- Other factors such as peripheral insulin resistance, carnitine deficiency, and hyperthyroidism may contribute to lipid abnormalities.
- In nephrotic syndrome, decreased effective plasma albumin circulation results in increased lipoprotein synthesis to maintain plasma oncotic pressure.

Pathophysiology of Hypercholesterolemia

LDL cholesterol normally circulates in the body for 2.5 days and subsequently binds to the LDL receptor on the liver cells, undergoes endocytosis and is digested. LDL is removed and synthesis of cholesterol by the liver is suppressed in the HMG-CoA reductase pathway. In FH, LDL receptor function is reduced or absent, and LDL circulates for an average duration of 4.5 days, resulting in significantly increased level of LDL cholesterol in the blood with normal levels of other lipoproteins. In mutations of Apo B, reduced binding of LDL particles to the receptor causes the increased level of LDL cholesterol. It is not known how the mutation causes LDL receptor dysfunction in mutations of PCSK9 and ARH.

Although atherosclerosis occurs to a certain degree in all people, FH patients may develop accelerated atherosclerosis due to the excess level of LDL. The degree of atherosclerosis approximately depends of the number of LDL receptors still expressed and the functionality of these receptors. In many heterozygous forms of FH, the receptor function is only mildly impaired, and LDL levels will remain relatively low. In the more serious homozygous forms, the receptor is not expressed at all.

Some studies of FH cohorts suggest that additional risk factors are generally at play when an FH patient develops atherosclerosis. In addition to the classic risk factors such as smoking, high blood pressure, and diabetes, genetic studies have shown that a common abnormality in the prothrombin gene (G20210A) increases the risk of cardiovascular events in patients with FH. Several studies found that a high level of lipoprotein (a) was an additional risk factor for ischemic heart disease. The risk was also found to be higher in patients with a specific genotype of the angiotensin converting enzyme (ACE).

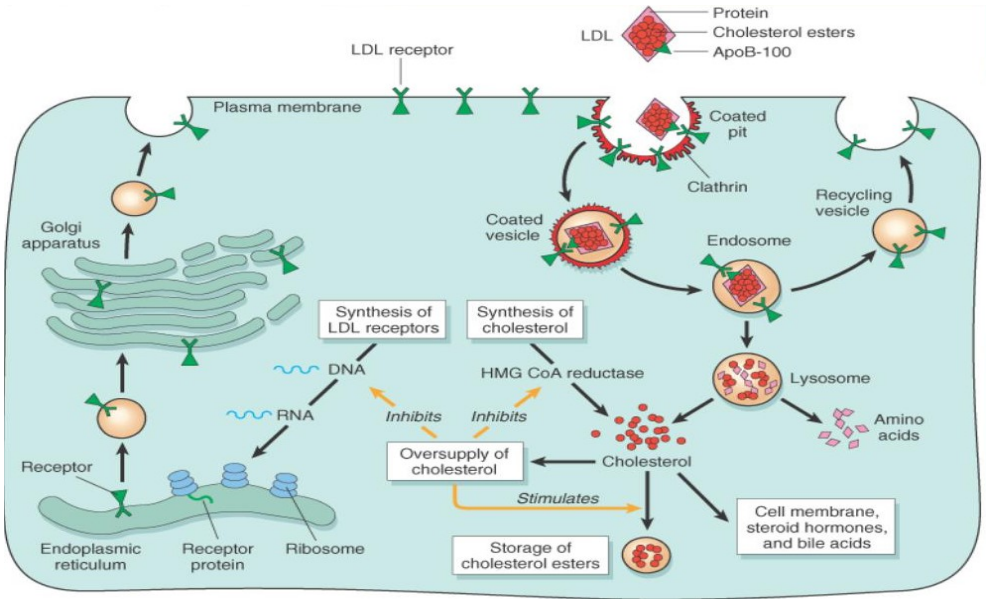


Figure 5.2: LDL receptor pathway and regulation of cholesterol metabolism

Pathophysiology of Hypertriglyceridemia

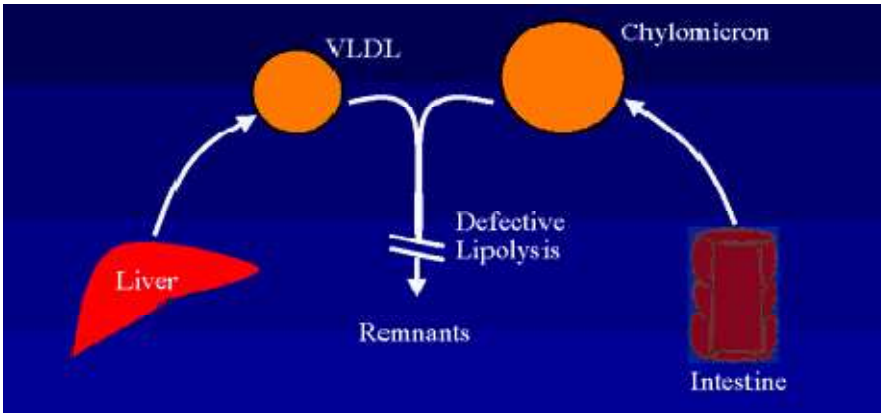


Figure 5.3: Development of Hypertriglyceridemia

Hypertriglyceridemia may be the result of two processes.

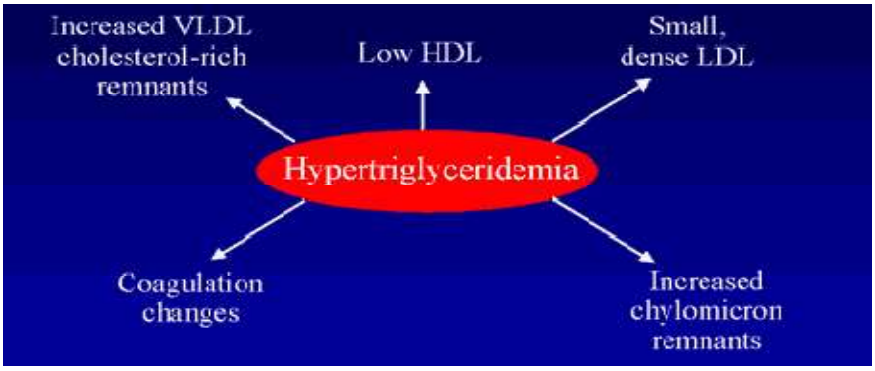


Figure 5.4: Possible atherogenic changes accompanying hyertriglyceridemia

- One process is the overproduction of VLDL by the liver in response to an increase in free fatty acids flowing to this organ.
- The other process is a defect in the lysis of VLDL triglycerides and chylomicrons by lipoprotein lipase. When lipoprotein lipase activity is deficient, triglycerides cannot be converted, hydrolyzed, or broken down, and the metabolism of Chylomicron and VLDL remnants may be delayed, as in type III hyperlipoproteinemias (dysbetalipoproteinemia).
- Plasma triglyceride levels less than 200 mg/Dl are classified as normal. There has been some debate about whether an elevated triglyceride level is an independent risk factor for atherosclerosis.
- Elevated triglycerides often reflect an increase in triglyceride-rich remnant lipoproteins that have atherogenic potential

TYPES OF HYPERLIPIDEMIA[85, 87, 89]

Hyperlipidemia can be broadly divided into:

On the basis of lipid type

1. Hypercholesterolemia
2. Hypertriglyceridemia

On the basis of causing factor

1. Primary (Familial)
2. Secondary (Acquired)

On the basis of lipid type

1. Hypercholesterolemia, in which there is a high level of cholesterol
2. Hypertriglyceridemia, in which there is a high level of triglycerides, the most

Primary (familial):

It is also called familial due to a specific genetic abnormalities. It may be monogenic: a single gene defect or polygenic: multiple gene defects, dietary and physical activity are caused due to it.

According to "fredrickson" classification, there are five types of primary hyperlipidaemia

Type I - Raised cholesterol with high triglyceride levels
Type II - High cholesterol with normal triglyceride levels
Type III - Raised cholesterol and triglycerides

Type IV - Raised triglycerides, atheroma, and raised uric acid

Type V – Raised cholesterol triglycerides

Table No.5.1: Fredrickson classification for primary hyperlipidemia

Hyperlipop-roteinemia		Synonyms	Defect	Increased lipoproten	Treatment	Occurrence
Type I	A	Buerger - Gruetzsyndrome or Familial	Decreased lipoprotein lipase (LPL)	Cm	Diet control	Very rare
	B	Hyperchylomicronemi -a	Altered ApoC2			
	C	Familial Apoprotein CII deficiency	LPL inhibitor in blood			
Type II	A	Familial hypercholesterolaemia	LDL receptor deficiency	LDL	Bile acid sequestrant, statins, niacin	Less common
	B	Polygenic hypercholesterolaemia	Decreased LDL receptor and increased ApoB	LDL and VLDL	Statins, niacin, fibrate	Commonest
Type III		Familial Dysbetalioproteinemia	Defectin ApoE2 synthesis	IDL Cm remnants	Fibrate, statins	Rare
Type IV		Familial Hypertriglyceridemia	Increased VLDL production and Decreased elimination	VLDL	Statins, niacin, fibrate	common
Type V		Familial combined hyperlipidaemia	Increased VLDL production and Decreased LPL	VLDL and LDL	Niacin, fibrate	Less common

Secondary (acquired):

Acquired hyperlipidemias (also called secondary dyslipoproteinemias) may mimic primary forms of hyperlipidemia and can have similar consequences. They

may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome.

The most common causes of acquired hyperlipidemia are:

- Diabetes Mellitus

Use of drugs such as diuretics, beta blockers and estrogens

Other conditions leading to acquired hyperlipidemia include:

- Hypothyroidism
- Renal Failure
- Nephrotic Syndrome
- Alcohol consumption.
- Some rare endocrine disorders and metabolic disorders

Signs and symptoms of Hyperlipidemia[91]

Person with hyperlipidemia usually does not have any noticeable symptoms in familial, or inherited, hyperlipidemia, but they are usually discovered during routine examination for atherosclerotic cardiovascular disease.

Symptoms may include:

- Chest pain (angina)
- Heart attack or stroke
- Atheromatous plaques in the arteries
- Abdominal Pain
- Xanthoma
- Xanthelasma of eyelid
- Swelling of organs such as liver, spleen or pancreas.
- Blockage of blood vessels in brain and heart.
- Higher rate of obesity and glucose intolerance
- High cholesterol or triglyceride levels
- Higher rate of obesity and glucose intolerance
- Arcus senilis
- Xanthomata

- Pancreatitis
- Numerous pimple-like lesions across their body

Causes of Hyperlipidemia[91]

- A diet rich in saturated fat and cholesterol increases blood cholesterol and triglyceride levels.
- Other disorders as obesity, diabetes mellitus (type 2), Kidney disease, Nephrotic Syndrome Obstructive Jaundice, Cushing's Syndrome and hypothyroidism increase the risk of hyperlipidemia.
- Smoking and not exercising may lead to hyperlipidemia.
- Excessive use of alcohol also increases the risk of hyperlipidemia.
- Certain drugs as steroids, Thiazide Diuretics, Ciclosporin, Retinoic Acid and β–blockers may cause hyperlipidemia.
- Hereditary factor is also one of the common causes for hyper-lipidemia.
- In some cases hyperlipidemia occurs during pregnancy (Familial hyperlipidemia stems from a genetic disorder).
- Lipoprotein lipase mutations
- Anorexia Nervosa
- Estrogen therapy

Complications of hyperlipidemia[92]

I. Atherosclerosis: It is a common disorder and occurs when fat, cholesterol and calcium deposits in the arterial linings. This deposition results in the formation of fibrous plaques. A plaque normally consists of three components: 1) atheroma which is a fatty, soft, yellowish nodular mass located in the centre of a larger plaque that consists of macrophages, which are cells that play a role in immunity; 2) a layer of cholesterol crystals; and, 3) calcified outer layer. Atherosclerosis is the leading cause of cardiovascular disease.

II. Coronary Artery Disease (CAD): Atherosclerosis is the major cause of CAD. It is characterized by the narrowing of the arteries that supply blood to the myocardium and results in limiting blood flow and insufficient amounts of oxygen to meet the needs of the heart. The narrowing may progress to the extent that the heart muscle would sustain damage due to lack of blood supply. Elevated lipid profile is correlated to the development of coronary atherosclerosis.

III. Myocardial Infarction (MI): MI is a condition which occurs when blood and oxygen supplies to the cardiac arteries are partially or completely blocked, resulting in damage or death of heart cells. The blockage is usually due to the formation of a clot in an artery. This condition is commonly known as heart attack. The studies show that one-fourth of survivors of myocardial infarction were hyperlipidemic.

IV. Angina Pectoris: Angina is not a disease but a symptom of an underlying heart condition. It is characterized by chest pain, discomfort or a squeezing pressure. Angina occurs as a result of a reduction or a lack of blood supply to a part or the entire heart muscle. Poor blood circulation is usually due to CHD when partial or complete obstruction of the coronary arteries is present.

V. Ischemic stroke or Cerebrovascular Accident (CVA): It occurs when blood circulation in part of the brain is blocked or diminished. When blood supply, which carries oxygen, glucose, and other nutrients, is disrupted, brain cells die and become dysfunctional. Usually, strokes occur due to blockage of an artery by a blood clot or a piece of atherosclerotic plaque that breaks loose in a small vessel within the brain. Clinical trials revealed that lowering of LDL and total cholesterol by 15% significantly reduced the risk of first stroke.

VI. Pancreatitis

Diagnosis of hyperlipidemia[93]

Hyperlipidemia typically shows no symptoms and can only be detected by a blood test. Screening for hyperlipidemia is done with a blood test called a lipid profile. According to National Cholesterol Education Program (NECP) screening, should start at age 20, and if the report is normal, it should be repeated at least every five years. Normal levels for a lipid profile are given table 5.2.

Skin assessment[89]

Xanthelasmas and xanthomas are fatty deposits under the skin surface commonly found in patients with metabolic disorders, such as elevated blood lipids and genetic disorders such as familial hypercholesterolemia. A biopsy will show fatty deposits.

Table No.5.2: Normal levels for a lipid profile

S.No	Test name	Normal values	Indicators
1	Total Cholesterol	Total Cholesterol: < 200 mg/dL (desirable) (< 180 optimal)	200-239 mg/dL = Borderline High (borderline risk for CHD) > 240 mg/dL =Hypercholesterolemia
2	Total Cholesterol for children	< 180 mg/dL	> 180 mg/dL may lead to premature Atherosclerosis
3	Triglyceride Levels	Less than 150 mg/dL	150-199 mg/dL is Borderline High 200-499 mg/dL is High 500 mg/dL or above is Very High
4	HDL Cholesterol	≥ 60 mg/dL is desirable	In general, HDL levels < 40 mg/dL increases risk for CHD. Women with levels < 47 mg/dL and men <37 mg/dL have increased risk.
5	LDL Cholesterol	< 100 mg/dL (optimal) 100-129 mg/dL (near optimal/above optimal)	130-159 mg/dL Borderline High 160-189 mg/dL High ≥190 mg/dL Very High
6	C-reactive Protein (CRP)	CRP < 1 mg/dl	CRP > 1 mg/dl (>10 mg/dl often seen) suggests inflammation
7.	VLDL cholesterol	normal range is 0–40 mg/Dl and recommended optimum range is 0–30 mg/dL	> 40 suggests can increase the risk of developing heart disease

Prevention of hyperlipidemia[93]

- Low fats and cholesterol diet should be taken.
- Eat foods high in soluble fiber such as oats, beans and certain fruits.
- Exercise regularly to maintain a healthy weight.

Controllable lifestyle changes are the best way to fight hyperlipidaemia. But when lifestyle changes fail to control the disease then treatment with cholesterol-lowering drugs is required.

Treatment of hyperlipidemia[87, 93]

Adult Treatment Panel (ATP) recommends two methods of treatment:

- Therapeutic lifestyle changes
- Drug therapy

Drugs used

1. **HMG-CoA reductase inhibitors (Statins):** Lovastatin, Simvastatin, Pravastatin, Atorvastin, Rosuvastin, Pitavastatin.

2. **Bile acid sequestrants (Resins):** Cholestyramine, Colestipol.

3. **Activate lipoprotein lipase (Fibric acid derivatives):** Clofibrate, Gemfibrozil, Benzafibrate, and Fenofibrate, Trilipix.

4. **Inhibit lipolysis and triglyceride synthesis:** Nicotinic acid.

5. **Others:** Ezetimibe, Gugulipid.

DRUG PROFILE

6. DRUG PROFILE

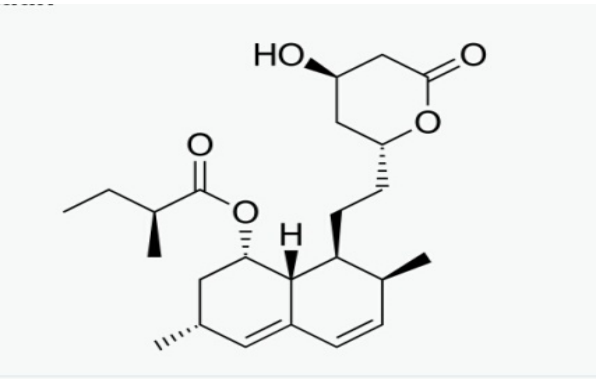
LOVASTATIN[86, 94]

Statins are compounds of natural origin that are biosynthesized as secondary metabolites of several filamentous fungi (*Aspergillus terreus*) and act as competitive inhibitors of HMG-CoA reductase. It is a member of the family of the substituted hexahydronaphthalene lactones

Category: Hypolipidemic agent, anticholesteremic agent, drug for CHD

PHYSIOCHEMICAL PROPERTIES

Chemical structure:



IUPAC name: (1S,3R,7S,8S,8aR)-8-{2-[2R,4R)-4-Hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethy-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2S)-2-methylbutanoate

CAS number: 75330-75-5

Description: A white, non-hygroscopic crystalline powder

Molecular weight: 404.547 g/ mol

Molecular formula: C_{24} H_{36} O_5

Melting point: 174.5^{0}C

Solubility: insoluble in water, in hexane; freely soluble in chloroform; soluble in dimethyl formamide; soluble in acetone,in acetonitrile and in methanol; sparingly soluble in alcohol.

Log P value: 4.26

Storage: stored in well-closed, light-resistant containers at 5-30^{0}C

PHARMACOKINETICS:

Absorption: Studies suggest that <5% of the oral dose reaches the general circulation as active inhibitors. Time to peak serum concentration is 2-4 hours. Lovastatin undergoes extensive first-pass metabolism so the availability of the drug in the system is low and variable.

Half life: 1-4 hours

Volume of distribution: Lovastatin is able to cross the blood-brain-barrier and placenta.

Protein binding: Lovastatin and its β-hydroxyacid metabolites are highly protein bound (>95%).

Metabolism: Lovastatin is hepatically metabolized in which the major active metabolites are the β-hydroxyacid of lovastatin, the 6'-hydroxy derivative, and two additional metabolites.

Elimination: Lovastatin undergoes extensive first-pass extraction in the liver, its primary site of action, with subsequent excretion of drug equivalents in the bile. 83% of the orally administered dose is excreted in bile and 10% is excreted in urine.

Clearance: Not Available

Toxicity: LD50 > 1000 mg / kg (orally in mice)

PHARMACODYNAMICS:

The primary cause of cardiovascular disease is atherosclerotic plaque formation. Sustained elevations of cholesterol in the blood increase the risk of cardiovascular disease. Lovastatin lowers hepatic cholesterol synthesis by competitively inhibiting HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthesis pathway via the mevalonic acid pathway. Decreased hepatic cholesterol levels causes increased uptake of low density lipoprotein (LDL) cholesterol and reduces cholesterol levels in the circulation. At

therapeutic doses, lovastatin decreases serum LDL cholesterol by 29-32%, increases high density lipoprotein (HDL) cholesterol by 4.6-7.3%, and decrease triglyceride levels by 2-12%. HDL cholesterol is thought to confer protective effects against CVD, whereas high LDL and triglyceride levels are associated with higher risk of disease.

MECHANISAM OF ACTION

Lovastatin is structurally similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase. Lovastatin is a prodrug that is activated *in vivo via* hydrolysis of the lactone ring to form the β-hydroxyacid. The hydrolyzed lactone ring mimics the tetrahedral intermediate produced by the reductase allowing the agent to bind to HMG-CoA reductase with 20,000 times greater affinity than its natural substrate. The bicyclic portion of lovastatin binds to the coenzyme A portion of the active site. Lovastatin lowers the hepatic cholesterol synthesis by compitatively inhibing the HMG – CoA reductase after hydrolysis of lactone ring in Lovastatin activated to beta hydroxy acid of lovastatin , that enzyme rate limiting step in cholesterol biosynthesis. That enzyme catalyzes the conversion of HMG CoA to mevalonate. Mevalonate is a required building block for cholesterol biosynthesis. After inhibition increased uptake of LDL-C, increased HDL-C, decreased TC level.

INDICATIONS AND USAGE [95]

Hyperlipidemia

It is indicated as an adjunct to diet for the reduction of elevated Total-C, LDL-C, Apo B, and TG, and to increase HDL-C in patients with primary hypercholesterolemia (heterozygous familial and non-familial) and mixed dyslipidemia (Fredrickson types IIa and IIb).

Coronary heart disease (CHD)

It is indicated to reduce the risk of myocardial infarction, Unstable angina, Coronary revascularization procedures, to slow the progression of coronary atherosclerosis in patients with coronary heart disease as part of a treatment strategy to lower Total-C and LDL-C to target levels.

DOSAGE

Usual adult dose for Hyperlipidemias; primary prophylaxis of coronary artery disease:

Immediate release formulation:

Initial dose: 20 mg orally once day with the evening meal.
Maintenance dose: 10 to 80 mg orally once a day or in 1 or 2 divided doses.

Extended release formulation:

Initial dose: 20, 40, 60 mg orally once a day at bedtime
Maintenance dose: 10 to 60mg orally given once a day at bedtime

Usual Child (10-17yr) dose for an adjunct with heterozygous familial hypercholesterolaemia:

Initial dose: 10-20 mg orally once daily
Maintenance dose: 10 to 40 mg once daily

DRUG INTERACTIONS:

- Increases risk of myopathy/rhabdomyolysis with amiodarone, colchicines, ranolazine, danazol, diltiazem and verapamil.
- Increases anticoagulant effect of warfarin.
- Increased risk of myopathy and rhabdomyolysis with concomitant CYP3A4 inhibitors (e.g. Nafezodone, Erythromycin, Telithromycin, HIV protease inhibitors, Itraconazole, ketoconazole, Posaconazole, Telaprevir), gemfibrozil, ciclosporin.

FOOD INTERACTIONS:

- Avoid alcohol.
- Avoid drastic changes in dietary habit.
- Avoid taking with grapefruit juice.
- Take with food, 50% increase in bioavailability when taken with food.

ADR[85]

GI disturbances, Headache, nausea, dizziness, insomnia, myopathy is the only serious reaction but rare, weakness, rhabdomyolysis , weakness, Myalgia, arthralgia, weight gain, blurred vision, rash, asymptomatic hepatic aminotransferase elevation, severre rhabdomyolysis with acute renal failure, hepatitis, pancreattis.

Rare:

Stevens - johnson syndrome, anaphylaxis, toxic epidermal necrolysis

CONTRAINDICATIONS

- Pregnancy and lactation
- Myopathy / rhabdomyolysis
- Active liver disease or unexplained persistent elevation of serum transaminase
- Concomitant use with CYP3A4 inhibitors

SPECIAL PRECAUTION[94]

History of liver disease, patients at risk of myopathy, alcoholism inadequately controlled hypothyroidism, severe renal impairment. Monitoring parameters: monitor creatine kinase (CK) periodically and LFT. Discontinue if there is significant persistent increase in CK level, serum aminotransferase level and evidence of myopathy.

MARKETED FORMULATION OF LOVASTATIN:

- Tab 10 mg Lochol - Microlab
- Tab 10 mg Lovex – Lupin laboratories
- Tab 20 mg Aztatin – Mon Pharma
- Tab 10 & 20 mg Rovacor – Ranbaxy laboratories
- Tab 20 mg Lovadac – Zydus cadila
- Tab 10, 20, 40, 60 mg Altopreve - Covis Pharma
- Tab 20, 40, 60 Mevacor- Merck

AVAILABLE MARKETED EXTENDED RELEASE PRODUCTS

Table No. 6.1: Marketed Lovstatin extended release products

NAME	DOSADE	STRENGTH	ROUTE	LABELLER
Altoprev	Tablet, extended release	60 mg/1	Oral	Physicians Total Care, Inc.
Altoprev	Tablet, extended release	20, 40, 60 mg/1	Oral	Covis Pharma
Altoprev	Tablet, extended release	20 mg/1	Oral	Shionogi

AVAILABLE MARKETED MIXTURE PRODUCTS

Table No. 6.1: Marketed mixture products of Lovastatin

NAME	DOSADE	STRENGTH	ROUTE	LABELLER
Advicor	Lovastatin (20 mg/1) + Niacin (50 0/750 mg/1)	Tablet, extended release	Oral	Physicians Total Care, Inc.
Advicor	Lovastatin (20 mg) + Niacin (500 mg)	Tablet, extended release Tablet, multilayer Tablet	Oral	Sepracor Pharmaceuticals Inc

EXCIPIENTS PROFILE

7. EXCIPIENTS PROFILE

7.1. EUDRAGIT RS 100[96]

1. Nonproprietary names

BP: Ammonio methacrylate copolymer (Type B); USP-NF: Ammonio methacrylate copolymer

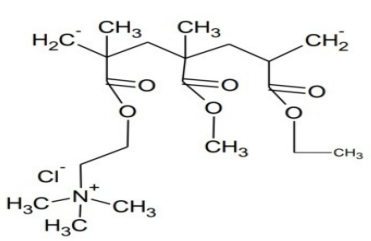
2. Synonyms

Eudragit, Eastacryl, Kollicoat MAE, Polymeicmethacrylates, Acryl-EZE

3. Chemical name

Poly (ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) 1:2:0:1

4. Structural formula



5. Molecular weight : >10000

6. Description: Eudragit RS 100 is ammonia methacrylate copolymers synthesized from acrylic acid and methacrylic acid esters. It occurs as fine granules with slight amine like odour.

7. Solubility: Eudragit RS 100 is soluble in acetone, alcohols, dichloromethane, ethyl acetate and are water insoluble

8. Functional category: Film forming agent, tablet binder, tablet diluents

9. Applications: Eudragit RS 100 is used to form water insoluble film coats for sustained release products. It is also used as binders in aqueous and organic wet granulation processes. They may also used in direct compression processes.

7.2. ETHYL CELLULOSE[96]

1. Nonproprietary names

BP: Ethyl cellulose PhEur: Ethyl cellulose USP-NF: Ethyl cellulose

2. Synonyms

Aquacoat ECD; Aqualon; E462; Ethocel; Surelease

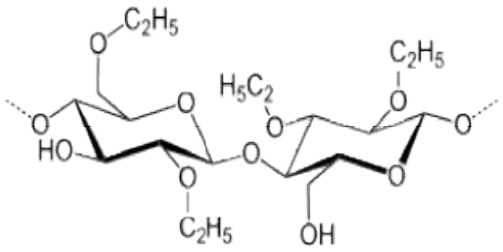
3. Chemical name

Cellulose ethyl ether [9004-57-3]

4. Empirical formula and molecular weight

Ethyl cellulose with complete ethoxyl substitution (DS=3) is $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$ where n can vary to provide a wide variety of molecular weights. Ethyl Cellulose, an ethyl ether of cellulose is a long chain polymer of b-anhydroglucose units joined together by acetal linkages.

5. Structural formula



6. Description

Ethyl cellulose is a tasteless, free flowing, white to light 0369tan coloured powder.

7. Solubility

- Ethyl cellulose is practically insoluble in glycerine, propylene glycol and water.
- Ethyl cellulose contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate and tetrahydrofuran, and in mixtures of aromatic hydrocarbon with ethanol (95%).
- Ethylcelluose that contain not less than 46.5% of ethoxy group is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and tolune.

8. Incomatibilities

Incompatible with paraffin wax and microcrystalline wax.

9. Functional category

Coating tablet, tablet binder, tablet filler and viscosity increasing agent.

10. Applications

- Ethyl cellulose is widely used in oral and topical pharmaceutical formulations.
- The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules.
- Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation.
- Ethyl cellulose dissolved in organic solvent can be used on its own to produce water- insoluble films.
- High-viscosity grades of ethylcellulose are used in drug microencapsulation.
- Used as Binder
- Ethylcellulose is used as a thickening agent in creams, lotions, or gels.
- Ethylcellulose has been studied as a stabilizer for emulsions.
- Microencapsulation 10.0–20.0 %
- Sustained-release tablet coating 3.0–20.0%
- Tablet coating 1.0–3.0 %
- Tablet granulation 1.0–3.0%

11. Stability and Storage conditions

- Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters.
- Ethyl cellulose is subjected to oxidative degradation in the presence of sunlight or UV light at elevated tempaerature. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230-340 nm range.
- Ethyl cellulose should be stored at a temperature not exceeding 32^0C in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

7.3 POLYVINYL ALCOHOL[96, 97]

1. Nonproprietary Names

PhEur: Poly (vinylisacetas) USP: Polyvinyl alcohol

2. Synonyms

Airvol; alcotex; elavanol; gelatol; gohsenol; lemol; mowiol; polyvinol; PVA; vinyl alcohol polymer.

3. Chemical name and CAS Registry number

Ethenol; Homopolymer [9002-89-5]

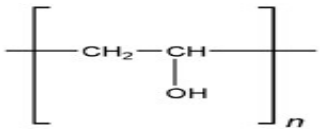
4. Empirical formula and molecular weight

$(C_2H_4O)_n$ 20000-200000 polyvinyl alcohol is a water-soluble synthetic polymer represented by the formula (C2H4O)n. the value of n for commercially available materials lies between 500 and 5000, equivalent to a molecular weight range of approximately 20000 -200000.

Commercially available grades of polyvinyl acohol

High viscosity -200 000
Medium viscosity -130 000
Low viscosity -20 000

5. Structural formula



6. Functional category

Coating agent; lubricant; stabilizing agent; Viscosity increasing agent.

7. Description

Odourless, white to cream coloured granular powder

8. Properties

Melting point: 228˚C for fully hydrolyzed grades;

180–190˚C for partially hydrolyzed grades.

9. Solubility

Soluble in water, slightly soluble in ethanol (95%), insoluble in organic solvents

10. Applications in pharmaceutical formulation or technology

- Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulation.
- It is used as a stabilizing agent for emulsions (0.25–3.0% w/v).
- Polyvinyl alcohol is also used as a viscosity-increasing agent for viscous formulations such as ophthalmic products.
- It is used in artificial tears and contact lens solutions for lubrication purposes.
- Used in sustained-release formulations for oral administration and in transdermal patches.
- Polyvinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution.
- As a surfactant for the formation of polymer encapsulated nanobeads
- It is used in textile sizing agent, in paper coatings and in release liner. As a water-soluble film useful for packaging.

Used in Emulsions - 0.5 % concentration,
Ophthalmic formulations - 0.25–3.00% concentration,
Topical lotions - 2.5% concentration.

11. Stability and Storage condition

Polyvinyl alcohol is stable when stored in a tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion resistant sealed containers. Preservatives may be added to the solution if extended storage is required. Polyvinyl alcohol undergoes slow degradation at 100^0C and rapid degradation at 200^0C; it is stable on exposure to light.

12. Safety

Polyvinyl alcohol is generally considered a nontoxic material. It is nonirritant to the skin and eyes at concentrations up to 10%; concentrations up to 7% are used in cosmetics.

MATERIALS

&

METHODS

8. MATERIALS AND METHODS

8.1. MATERIALS USED IN FORMULATION

Table No.8.1: The list of drug and excipients, their manufacturer and role in formulation

INGREDIENTS	MANUFACTURER/SUPPLIER	ROLE IN FORMULATION
Lovastatin	Micro lab Pvt. Ltd, Hosur	Active ingredient
Eudragit RS 100	Evonik India Pvt, Ltd. Mumbai	Polymer
Ethyl cellulose	Micro lab Pvt. Ltd, Hosur	Polymer
Poly vinyl alcohol	Green moon biochem, Chennai.	Stabilizing agent
Dichloromethane	Micro Fine chemical	Solvent
Methanol	Micro Fine chemical, Chennai.	Solvent
Potassium dihydrogen phosphate	Lab chemicals, Chennai.	Reagent
Sodium hydroxide	Lab chemicals, Chennai.	Reagent

8.2 EQUIPMENTS / INSTRUMENTS USED IN THE STUDY

Table No. 8.2: Equipments used in the formulation and evaluation of NPs

S.NO	EQUIPMENTS	MANUFACTURERS/SUPPLIERS
1	Electronic weighing balance	Mc Dalal, Chennai
2	Fourier Transform Infrared Spectroscopy	Nicolet, India
3	UV-Visible Spectrophotometer	Shimadzu, Japan Jasco,
4	Ultra Sonicator	Lark, Chennai
5	Magnetic Stirrer	Remi Instruments, Mumbai
6	High speed cooling centrifuge	Remi Instruments, Mumbai
7	Scanning electron microscope	Hitachi X650 Tokyo, Japan
8	Particle size analyzer	Malvern Instrument, UK
9	pH Meter	Mc Dalal, Chennai
10	Freeze dryer	Mc Dalal, Chennai
11	Zeta sizer	Malvern Instrument, UK
12	Stability chamber	Remi che-6 plus
13	pH meter	Symchrony.India.
14	Hot air oven	Mc Dalal, Chennai
15	Disintegration test apparatus	Duralab
16	Dissolution test apparatus	Lab india DS4000
17	Digital melting Point apparatus	Guna

METHODOLOGY

8.3. PREFORMULATION STUDIES

8.3.1. DRUG-EXCIPIENT COMPATABILITY STUDY[98]

The drug and excipients selected for the formulation were evaluated for physical and chemical compatibility studies.

8.3.1.1. PHYSICAL COMPATIBILITY STUDY

100 mg each of powder drug, polymer, PVA were weighed. Individual drug, Polymer (Ethyl Cellulose and Eudragit RS 100), PVA along with admixture of drug and excipients in airtight screw cap amber coloured vials were kept at room temperature as well as 40^{o}C±2^{o}C / 75% ± 5% RH for 30 days. Change in colour was observed after 10, 20, 30 days.

8.3.1.2. CHEMICAL COMPATIBILITY STUDY[99, 100]

FTIR was used to find whether any kind of interaction between drug and polymer. Infrared spectroscopy was conducted using FT-IR spectrophotometer and the spectrum was recorded in the wave number region of 4000 to 400 cm^{-1}. The procedure consist of dispersing the sample (drug alone, Mixture of drug and excipients and the optimized formulation) in potassium bromide and compressed into discs by applying a hydraulic pressure. The pellet was placed in light path and spectrum was recorded.

8.3.2. DETERMINATION OF MELTING POINT[44]

Small amount of drug was loaded in a capillary tube where one end of capillary tube was closed and kept in melting point apparatus and temperature was noted when drug melts.

8.3.3. DETERMINATION OF LAMBDA MAX[45, 73]

100 mg of Lovastatin was weighed and transferred to 100 ml of volumetric flask. The drug was dissolved in 20 ml of methanol and volume was made up to 100 ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000 µg/ml (stock solution I). 10 ml of this stock solution was again diluted with phosphate buffer pH

6.8 up to 100 ml to obtain a solution of 100 µg/ml (Stock solution II). From stock solution-II, 10 ml was pipette out in 100 ml volumetric flask. The volume was made up to 100 ml using phosphate buffer pH 6.8 get a concentration of 10 µg/ml. This solution was then scanned at 200-400nm in UV-Visible spectrophotometer to attain the absorption maximum (λ-max).

8.3.4. STANDARD CURVE FOR LOVASTATIN[67]

100 mg of Lovastatin was weighed and transferred to 100 ml of volumetric flask. The drug was dissolved in 10 ml of methanol and volume was made up to 100 ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000 µg/ml (stock solution I). 10 ml of this stock solution was again diluted with phosphate buffer pH 6.8 up to 100 ml to obtain a solution of 100 µg/ml (Stock solution II). From stock solution II 2,4,6,8 10 ml were transferred to series of 100 ml volumetric flasks. The volume was made up with phosphate buffer pH 6.8. The absorbance of these solutions was measured at 238 nm against blank.

8.3.5. SOLUBILITY STUDIES OF PURE LOVASTATIN[77]

Solubility of Lovastatin pure drug was tested in distilled water and phosphate buffer pH 6.8. An excess amount of Lovastatin pure drug was added in 20 ml of the pertinent media. The mixtures were stirred in a mechanical shaker at speed of 50 rpm for 24 hours and the temperature was maintained at 37±0.5^{0}C. Visual inspection was carefully made to ensure there were excess lovastatin solids in the mixture, indicating saturation had been reached. Then the mixtures were filtered using 0.45µm micropore filter and filtrates were suitably diluted with same media. The absorbance of the solution was measured at 238nm in UV-Visible spectrophotometer.

8.4. FORMULATION DEVELOPMENT [58]

Formulation of Lovastatin Nanosponges

Lovastatin Nanosponges were prepared by Emulsion solvent evaporation method using polymers like Eudragit RS 100 and Ethyl cellulose at different drug to polymer ratios (1:1, 1:2, 1:3, 1:4, and 1:5).

Procedure to formulate Lovastatin Nanosponges

- Lovastatin Nanosponges were prepared by Emulsion solvent evaporation method. Two different polymers were used in the formulation. Eudragit RS 100 (EUD) and Ethyl cellulose (EC) were the Polymers used.
- External phase: Polyvinyl alcohol in Distilled water was used as the aqueous phase.
- Internal phase: The Drug was dissolved in the required solvent (Dichloromethane) and the Polymers (1:1, 1:2, 1:3, 1:4, and 1:5) was dissolved in Dichloromethane. The Drug solution was poured into the polymer solution and the mixture was shaken well.
- Then the Drug polymer mixture was poured into the aqueous phase and the mixture was subjected to homogenization using High speed homogenizer at 1500 rpm for 2 hours at room temperature.
- The formed Nanosponges were centrifuged by high speed cooling centrifuge and the residue was freeze dried.

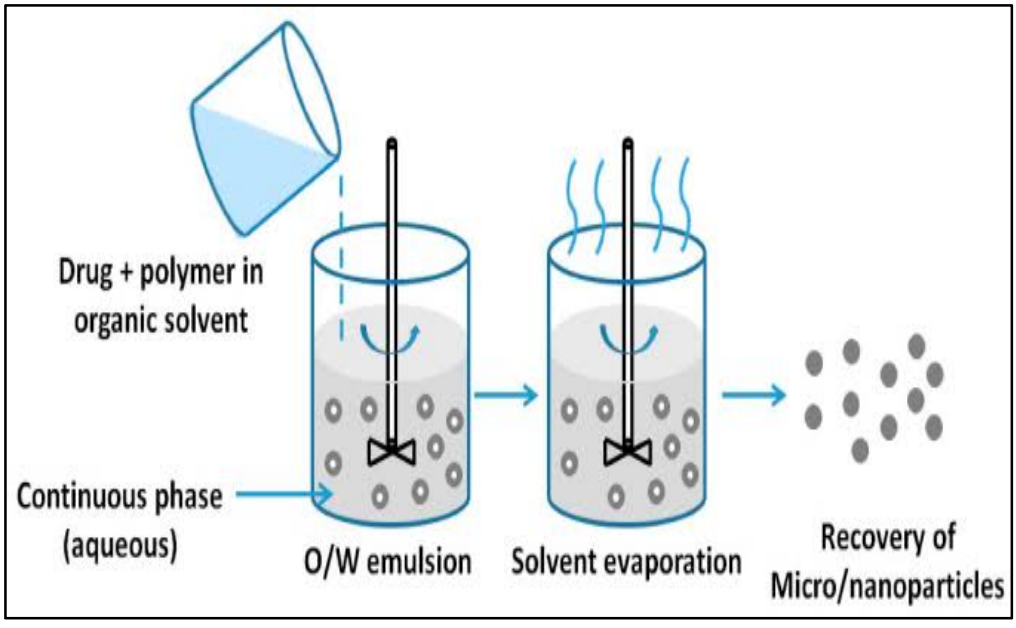


Figure 8.1: Schematic Representation of Preparation of Nanosponges

Table No. 8.3: Composition of Lovastatin Nanosponges

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Drug: polymer ratio	1:1	1:2	1:3	1:4	1:5	1:1	1:2	1:3	1:4	1:5
Lovastatin (mg)	500	500	500	500	500	500	500	500	500	500
Eudragit RS 100 (g)	0.5	1	1.5	2	2.5	-	-	-	-	-
Ethyl cellulose (g)	-	-	-	-	-	0.5	1	1.5	2	2.5
Polyvinyl alcohol (g)	1	1	1	1	1	1.5	1.5	1.5	1.5	1.5
Dichloromethane (ml)	20	20	20	20	20	20	20	20	20	20
Distilled water (ml)	100	100	100	100	100	100	100	100	100	100

8.5. EVALUATION OF LOVASTATIN LOADED NANOSPONGES

8.5.1. PRODUCTION YIELD[30]

Percentage yield can be determined by calculating the initial weight of raw materials and the finally obtained weight of Nanosponges. Percentage yield can be calculated by using the formula.

$$\text{Production yield} = \frac{\text{Practical mass of Nansponges}}{\text{Theoretical mass (drug+polymer)}} \text{ X } 100$$

8.5.2. DRUG ENTRAPMENT EFFICIENCY[40, 73, 100]

The drug entrapment efficiency of Nanosponges was determined spectrophotometrically (λ_{max}=238 nm). A sample of Lovastatin Nanosponge was mixed in methanol and made upto 100 ml with phosphate buffer (pH 6.8) and kept it for overnight. The drug content was determined and expressed as actual drug

content in Nanosponges. The percentage entrapment efficiency (%EE) is calculated by following formula:

$$\text{Percentage entrapment efficiency} = \frac{\text{Actual drug content in Nanosponges}}{\text{Theoretical drug content}} \text{ X } 100$$

8.5.3. SOLUBILITY STUDIES OF LOVASTATIN NPs[76]

Solubility of Lovastatin Nanosponges formulations were tested in distilled water and phosphate buffer pH 6.8. An excess amount of Lovastatin Nanosponge formulation was added in 20 ml of the pertinent media. The mixtures were stirred in a mechanical shaker at speed of 50 rpm for 24 hours and the temperature was maintained at 37±0.5^{0}C. Visual inspection was carefully made to ensure there were excess Lovastatin NPs in the mixture, indicating saturation had been reached. Then the mixtures were filtered using 0.45µm micropore filter and filtrates were suitably diluted with same media and the absorbance of the solution measured at 238 nm in UV Visible spectrophotometer.

8.5.4. *IN VITRO* DRUG RELEASE STUDIES[65]

The *in vitro* release of Lovastatin from Nanosponges was evaluated using USP Type-II (Paddle) dissolution test apparatus. Lovastatin loaded Nanosponges were filled in capsule and placed in a dissolution jar containing 900ml of Phosphate buffer pH 6.8 as dissolution medium maintained at 37±0.5^{0}C and rotated at 50 rpm. 10 ml of samples were withdrawn at predetermined intervals upto 12 hrs and replaced with equal amount of phosphate buffer pH 6.8 for further dissolution testing. The absorbance was determined spectrophotometrically at 238 nm.

8.5.5. MORPHOLOGY OF NANOSPONGE BY SCANNING ELECTRON MICROSCOPY[49]

The Surface Morphology of the Nanosponges can be measured by SEM. Scanning electron microscopy was used to analyze particle size, shape and surface morphology of Nanosonges. The sample was mounted directly onto the SEM sample holder using double sided sticking tape and images were recorded at different magnifications at acceleration voltage of 10 kV using scanning electron microscope.

8.5.6. PARTICLESIZE AND POLYDISPERSITY[19, 61]

Particlesize (z-average diameter) and polydispersity index (as a measure of particle size distribution) of Lovastatin loaded Nanosponge dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 Nano S (Malvern instruments, UK) at 25°C.

Prior to measurements all samples were diluted using ultra–purified water to yield a suitable scattering intensity. The diluted nanosponge dispersion was poured into disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement.

Monodisperse samples have a lower PDI value, whereas higher PDI value indicates a wider particle size distribution and the polydisperse nature of the sample can be calculated by following equation:

PDI = d/d avg

Where**,**

d is the width of distribution denoted by **SD,**

d avg is the average particle size denoted by **MV(nm)** in particle size data sheet.

Table No. 8.4: Polydispersity Index

Polydispersity Index	Type of dispersion
0-0.05	Monodisperse standard
0.05-0.08	Nearly monodisperse
0.08-0.7	Mid range polydisperse
> 0.7	Very polydisperse

8.5.7. ZETA POTENTIAL[43, 49]

The ZP is a determinant of the electric charge on the surface of the particles. The physical stability of colloidal systems is indicated by ZP values. The ZP values were assessed by determining surface charge on the Lovastatin loaded Nanosponges using Malvern Zetasizer. 1ml of sample of Lovastatin suspension was filled in clear disposable zeta cell, without air bubble within the sample, the system was set at 25°C temperature and results recorded. The more negative zeta potential, greater the net charge of particles and more steady the Nanosponges preparation.

8.6. PREFORMULATION STUDY OF OPTIMIZED NANOSPONGES

8.6.1. FLOW PROPERTY MEASUREMENTS[74, 101]

The flow properties of powders are critical for an efficient tabletting and capsule filling operation. A good flow of the powder or granules is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablets and capsules. The flow property measurements include bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio. The flow properties of Nanosponges are determined.

8.6.1.1. BULK DENSITY (ρb)

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and initial weight was noted. This initial volume was called the bulk volume. From this the bulk density was calculated according to the formula mentioned below. It is expressed in g/ml and is given by

$$\rho b = M/V_b$$

Where, **M** and V_b are mass of powder and bulk volume of the powder respectively.

8.6.1.2. TAPPED DENSITY (ρ_t)

It is the ratio of weight of the powder to the tapped volume of powder. The powder was introduced into a measuring cylinder with the aid of funnel and tapped

for 300 times on a wooden surface at a 2 sec interval and the volume attained is the tapped volume.

$$\rho t = m/Vt$$

Where, **M** and **Vt** are mass of powder and tapped volume of the powder respectively.

8.6.1.3. ANGLE OF REPOSE (θ)

The flow properties were characterized in terms of angle of repose, Carr's index and Hausners's ratio. For determination of angle of repose, the drug and the blend were poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above hard surface. The drug or the blends was poured till the time when upper tip of the pile surface touched the lower tip of the funnel. Angle of repose was calculated using the following equation.

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

Where **h**= height of pile in cm; **r** = radius of pile in cm.

8.6.1.4. CARR'S INDEX OR % COMPRESSIBILITY

It indicates powder flow properties. It is measured for determining the relativ importance of interparticulate interactions. It is expressed in percentage and is given by

$$CI = \frac{\rho t - \rho b}{\rho t}$$

Where ρ_t and ρ_b are tapped density and bulk density respectively.

8.6.1.5. HAUSNER'S RATIO

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

$$HR = \rho_t / \rho_b$$

Where ρ_t and ρ_b are tapped density and bulk density respectively.

8.6.2. POROSITY (%)

Porosity is defined as the ratio of the void volume to the bulk volume of a powder packing. It is calculated using true density value obtained from liquid displacement. It gives total porosity since the void space determined takes into account the intra particle space i.e pores and cracks within the particles. It is also given as.

$$\varepsilon = 1 - (\text{Bulk density} / \text{True density}) \times 100$$

Table No.8.5: Angle of Repose, Compressibility Index and Hausner's ratio

Flow property	Angle of repose (θ)	Compressibility Index (%)	Hausner's ratio
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-55	26-31	1.35-1.45
Very poor	56-65	32-37	1.46-1.59
Very very poor	>65	>38	>1.60

8.7. OPTIMIZED NANOSPONGES FILLED IN HARD GELATIN CAPSULES

The optimized Lovastatin loaded Nanosponges were filled into hard gelatin capsule each containing 40 mg of Lovastatin.[98]

8.8. EVALUATION OF CAPSULES

8.8.1. UNIFORMITY OF WEIGHT[102]

Intact capsule were weighed. The capsules were opened without losing any part of the shell and contents were removed as completely as possible. The shell was washed with ether and the shell allowed to stand until the odour of the solvent was no longer detectable. The empty shell was weighed. The procedure was repeated with a further 19 capsules. The average weight was determined. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation and none deviates by more than twice that percentage.

Table No.8.6.Uniformity of weight (I.P. Standard)

Average weight of capsule contents	Percentage Deviation
Less than 300 mg	±10
300 mg or more	±7.5

8.8.2. DISINTEGRATION TEST[102]

One capsules introduced in to each tube of the disintegration test apparatus. A disc may be added if necessary. The basket rack assembly is suspended in the beaker containing the liquid medium. The apparatus is operated and the time for disintegration is noted.

Table No.8.7: Disintegration time (I.P. Standard)

Type of capsules	Medium	Disintegration time
Hard capsules	Water	30 min
Soft capsules	Water	60 min
Enteric capsules	0.1 M Hydrochloric acid	Do not disintegrate for 2 hrs
	Mixed phosphate buffer pH 6.8	One hour

8.8.3. DRUG CONTENT[102]

Five capsules were selected randomly and the average weight was calculated. An amount of powder was equivalent to 40 mg of Lovastatin was made upto 100 ml with phosphate buffer pH 6.8. It was kept overnight. 1 ml of solution was diluted to 50 ml using phosphate buffer pH 6.8 in separate standard flask. The absorbance of solution was recorded at 238 nm.

8.8.4. *IN VITRO* DRUG RELEASE STUDY[65]

The *in vitro* release of Lovastatin from Nanosponge was evaluated using USP Type-II (Paddle) dissolution test apparatus. Lovastatin loaded Nanosponges were filled in capsule and placed in a dissolution jar containing 900ml of Phosphate buffer pH 6.8 as dissolution medium maintained at 37±0.5^{0}C and rotated at 50 rpm. 10 ml of samples were withdrawn at predetermined intervals upto 12 hrs and replaced with equal amount of phosphate buffer pH 6.8 for further dissolution testing. The absorbance was determined spectrophotometrically at 238 nm.

8.9. RELEASE KINETICS OF THE OPTIMIZED FORMULATIONS[6, 46, 103]

To study the *in vitro* release kinetics of the optimized formulation, data obtained from dissolution study were plotted in various kinetics models.

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), Korsmeyer-Peppas model (Log Cumulative percent drug release versus log time) and Hixson Crowell model(cube root of log cumulative percentage of drug remaining vs. log time) were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best-fit model was decided.

Zero order equation:

The zero order release can be obtained by plotting cumulative % percentage drug released vs. time in h

$$C=K_0t$$

Where, $\mathbf{K_0}$ = Zero order constant

t = time in h

Application: It is used to describe the drug dissolution of several types of modified release Pharmaceutical dosage forms, as in the case of some transdermal systems, as well as tablets with low soluble drugs in coated forms, osmotic systems, etc.

First order reaction:

The graph was plotted as % cumulative drug release vs. time in h

$$\mathbf{Log\ C = Log\ C_0 - Kt / 2.303}$$

Where, C_0 = initial concentration of drug,

K = First order

t = time in h

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

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

Higuchi kinetics:

It was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that

- Initial drug concentration in the is much higher than drug solubility.
- Drug diffusion takes place only in one dimension (edge effect must be negligible).
- Drug particles are much smaller than system thickness.
- Swelling and dissolution are negligible.
- Drug diffusivity is constant and Perfect sink conditions are always attained in the release environment.

The graph was plotted with % cumulative drug release vs. square root of time.

$$Q = Kt^{1/2}$$

Where, **K** = constant reflecting design variable system (differential rate constant)

t = time in h

The drug release rate is inversely proportional to the square root of time.

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and tablets with water soluble drugs.

Hixson and Crowell erosion equation:

To evaluate the drug release with changes in the surface area and the diameter of the particles, the data were plotted using the Hixson and Crowell rate equation. The graph was plotted by cube root of % drug remaining vs. time in h.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}t$$

Where, Q_0 = Initial amount of drug

Q_t = Amount of drug released in time t

K_{HC} = Rate constant for Hixson Crowell equation

Korsmeyer- peppas equation:

Korsmeyer et al.(1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model.

To evaluate the mechanism of drug release, t was further plotted in korsmeyer-peppas equation as log cumulative % of drug released vs log time

$$M_t / M_\alpha = Kt^n$$

Where, M_t / M_α = Fraction of rug released at time t

t = Release time

K = kinetics constant (instructing structural and geometric characteristic of the formulation)

N= Diffusional exponent indicative of the mechanism of drug release

In this model, the value of n characterizes the release mechanism of drug as described in the below table.

Table No.8.8: Diffusion exponent and solute release mechanism

Diffusion Coefficient	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45<n<0.89	Anamolous (non-fickian diffusion)
0.89	Case II transport
n>0.89	Super case II transport

8.10. STABILITY STUDIES[45, 49]

The purpose of stability testing was to provide evidence on how the quality of a drug substance or Nanosponges varies with time under influence of varies environmental factors such as temperature, humidity and light.

Stability studies were carried out as per ICH guidelines. The optimized Lovastatin Nanosponges formulations were placed in airtight screw cap, amber coloured vials and kept under accelerated conditions (temperature 40°C±2°C and RH 75±5%) using stability chamber and refrigerated temperature (4±2^{0}C) for the period of 3 month. The samples were withdrawn at predetermined intervals and evaluated for their physical appearance, entrapment efficiency, drug content and *in vitro* drug release.

RESULTS

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DISCUSSION

9. RESULT AND DISCUSSION

9.1. PRE-FORMULATION STUDIES

9.1.1. Drug-Excipients compatibility study

The optimization of a formulation can be done only after a thorough investigation of its physic chemical properties of the drug and excipients. The drug and the polymer must be compatible for a successful formulation

9.1.1.1. Physical compatibility study

Table No.9.1: Physical compatibility study of Drug and Excipients

S.No.	Drug and Excipients	Description and Condition						
		Initial	At room temperature (in days)			At 40ºC ± 2ºand 75% RH± 2% (in days)		
			10	20	30	10	20	30
1	Lovastatin	White crystalline Powder	NC	NC	NC	NC	NC	NC
2	EUD RS 100	White Coloured Granules	NC	NC	NC	NC	NC	NC
3	EC	White Coloured powder	NC	NC	NC	NC	NC	NC
4	PVA	White Coloured granular powder	NC	NC	NC	NC	NC	NC
5	LOV + EC	White crystalline Powder	NC	NC	NC	NC	NC	NC
6	LOV + EUD RS 100	White crystalline Powder	NC	NC	NC	NC	NC	NC
7.	LOV + PVA	White crystalline Powder	NC	NC	NC	NC	NC	NC

*NC- No Change

Inference

The Physical compatibility is shown in table 9.1**.** They were evaluated for 10, 20 and 30 days at room temperature and at 40˚C±2˚C/75±5% Relative Humidity. There was no change of colour. Therefore the drug and excipients are physically compatible with each other. The excipients which are compatible with the drug were selected for formulation.

9.1.1.2. CHEMICAL COMPATIBILITY STUDY

FT-IR spectroscopic studies

FT-IR spectroscopy gives the possible information about the interaction between the drug and polymers. The results are follows

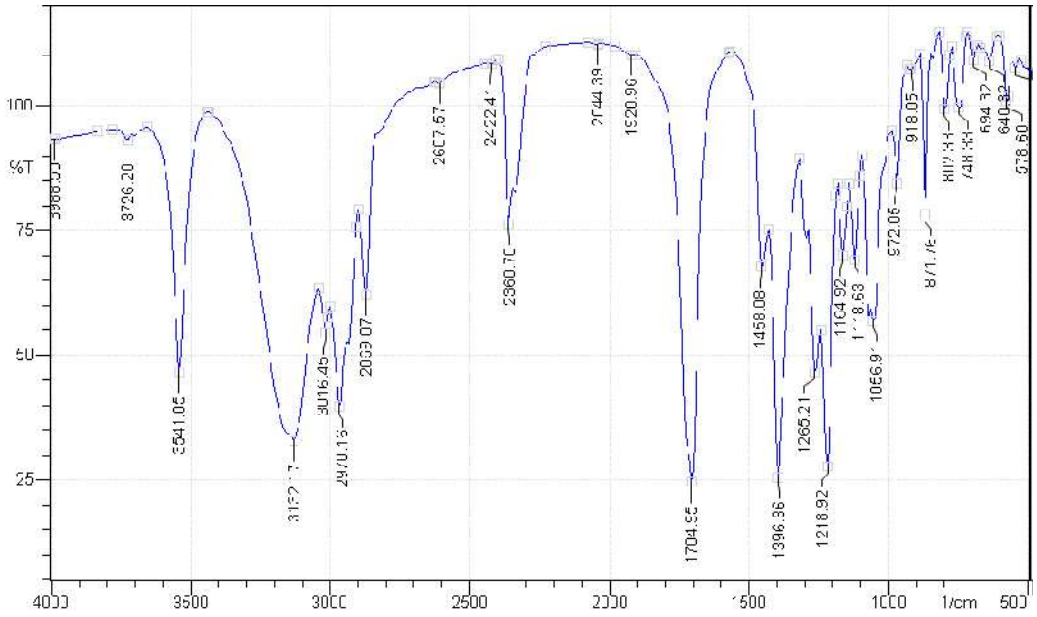


Figure 9.1: FT-IR spectrum of Lovastatin

Table No.9.2: FT-IR spectral interpretation of Lovastatin

Wave number (cm^{-1})	Types of vibrations	Functional group
3541.05	O-H Stretching	Presence of hydroxyl group
3132.17	C-H Stretching	Olefenic C-H group
3016.45	C-H Stretching	Methyl C-H group
1704.95	Lactone, ester carbonyl stretching	Lactone, ester group
1218.92	Ester C-O-C bending	Ester group

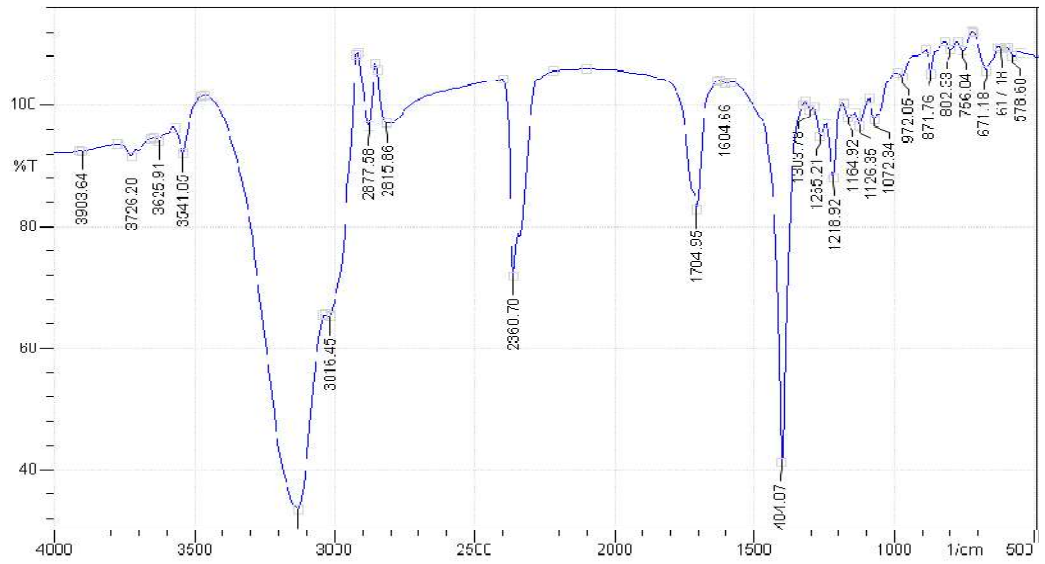


Figure 9.2: FT-IR of Lovastatin and Eudragit RS 100 admixture

Table No.9.3: FT-IR spectral interpretation of Lovastatin and Eudragit RS 100 admixture

Wave number (cm^{-1})	Types of vibrations	Functional group
3541.05	O-H Stretching	Presence of hydroxyl group
3132.17	C-H Stretching	Olefenic C-H group
3016.45	C-H Stretching	Methyl C-H group
1704.95	Lactone, ester carbonyl stretching	Lactone, ester group
1218.92	Ester C-O-C bending	Ester group

Inference

The peak observed in the FT-IR spectrum for Lovastatin and Eudragit RS 100 was shown in figure 9.2. It shows no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and Eudragit RS 100.[71, 99]

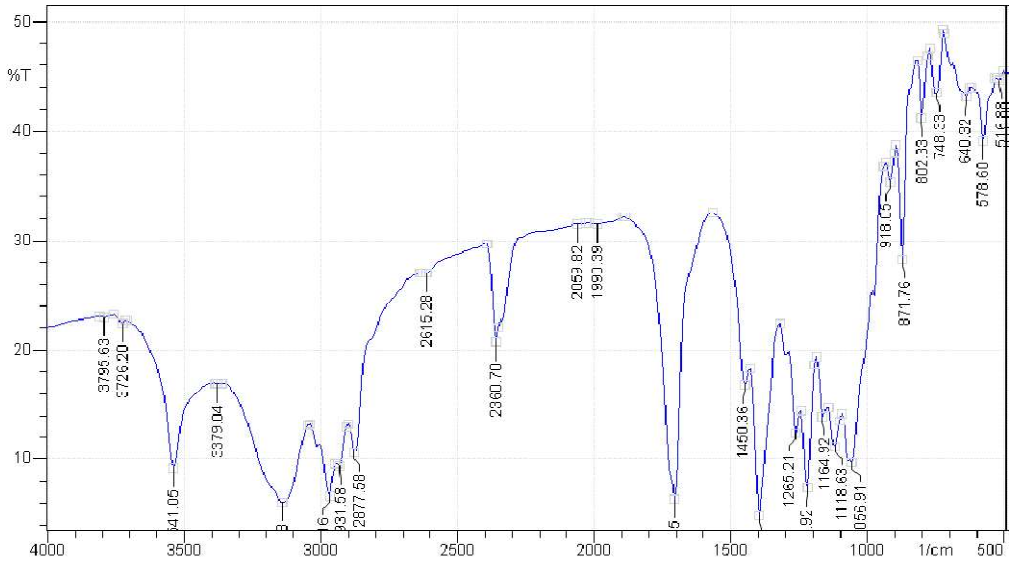


Figure 9.3: FT-IR of Lovastatin and Ethyl cellulose admixture

Table No.9.4: FT-IR spectral interpretation of Lovastatin and Ethyl cellulose admixture

Wave number (cm^{-1})	Types of vibrations	Functional group
3541.05	O-H Stretching	Presence of hydroxyl group
3139.88	C-H Stretching	Olefenic C-H group
2970.16	C-H Stretching	Methyl C-H group
1704.95	Lactone, ester carbonyl stretching	Lactone, ester group
1218.92	Ester C-O-C bending	Ester group

Inference

The peak observed in the FT-IR spectrum for Lovastatin and Ethyl cellulose was showed in figure 9.3. It shows no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and Ethyl cellulose.[99]

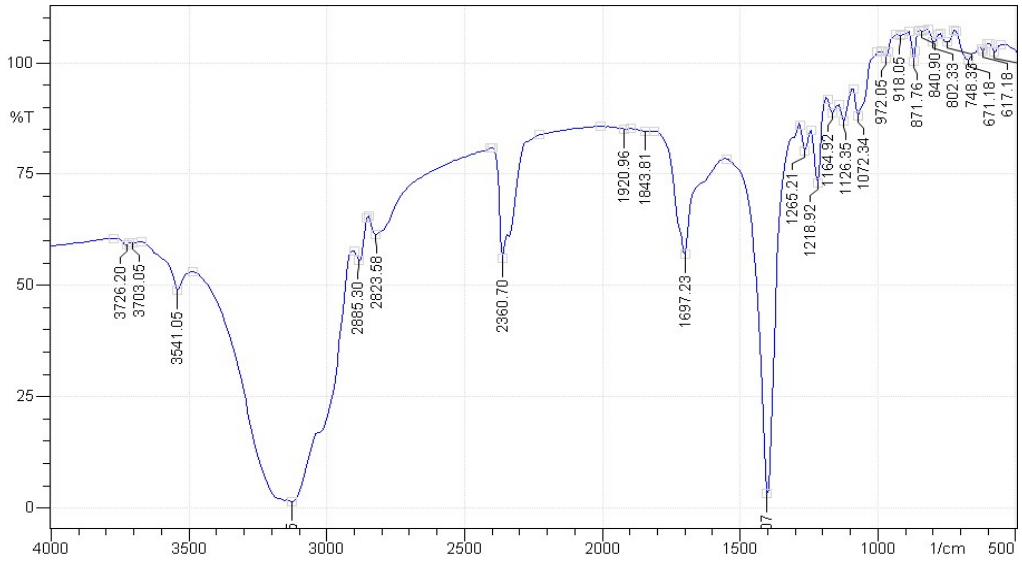


Figure 9.4: FT-IR of Lovastatin and Polyvinyl alcohol admixture

Table No.9.5: FT-IR spectral interpretation of Lovastatin and Polyvinyl alcohol admixture

Wave number (cm^{-1})	Types of vibrations	Functional group
3541.05	O-H Stretching	Presence of hydroxyl group
3132.17	C-H Stretching	Olefenic C-H group
1704.95	Lactone, ester carbonyl stretching	Lactone, ester group
1218.92	Ester C-O-C bending	Ester group

Inference

The peak observed in the FT-IR spectrum for Lovastatin and Polyvinyl alcohol was shown in figure 9.4. It shows no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and polyvinyl alcohol.[99]

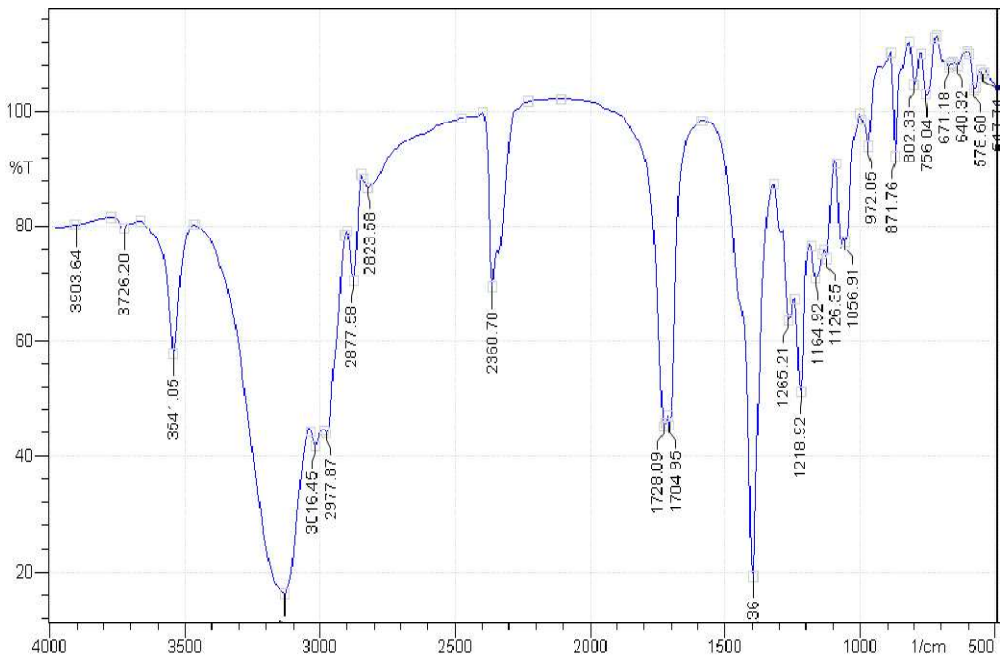


Figure 9.5: FT-IR of Optimized Formulation F3

Table No.9.6: FT-IR spectral interpretation of Optimized Formulation F3

Wave number (cm^{-1})	Types of vibrations	Functional group
3541.05	O-H Stretching	Presence of hydroxyl group
3132.17	C-H Stretching	Olefenic C-H group
3016.45	C-H Stretching	Methyl C-H group
1704.95	Lactone, ester carbonyl stretching	Lactone, ester group
1218.92	Ester C-O-C bending	Ester group

Inference

From the FT-IR spectra, it is clearly evident that the optimized formulation F3 showed the presence of characteristics bands Lovastatin. This indicates the absence of chemical interaction between the Lovastatin and the excipients.[99]

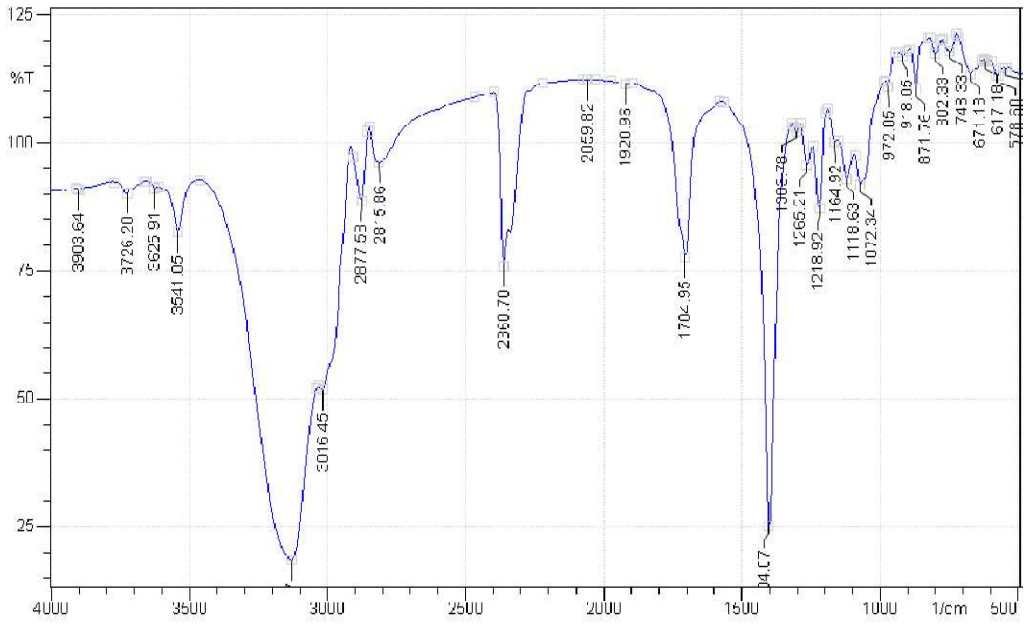


Figure 9.6: FT-IR of Optimized Formulation F8

Table No.9.7: FT-IR spectral interpretation of Optimized Formulation F8

Wave number (cm^{-1})	Types of vibrations	Functional group
3541.05	O-H Stretching	Presence of hydroxyl group
3132.17	C-H Stretching	Olefenic C-H group
3016.45	C-H Stretching	Methyl C-H group
1704.95	Lactone, ester carbonyl stretching	Lactone, ester group
1218.92	Ester C-O-C bending	Ester group

Inference

From the FT-IR spectra, it is clearly evident that the optimized formulation F8 showed the presence of characteristics bands Lovastatin. This indicates the absence of chemical interaction between the Lovastatin and the excipients.[99]

9.3.2. DETERMINATION MELTING POINT

Melting point of Lovastatin was observed for quality determination, it matches with standard value[86] and the values are given in the table 9.8.

Table No.9.8: Melting point of drug

Drug	Standard value	Experimental value
Lovastatin	174.5^0C	$174\text{-}175^0$C

9.3.3. DETERMINATION OF LAMBDA MAX FOR LOVASTATIN

The maximum absorbance of the Lovastatin was studied. The maximum absorbance of the Lovastatin was found to be 238 nm as shown in figure 9.7. Hence the wavelength of 238 nm was selected for analysis of drug in dissolution media.[86]

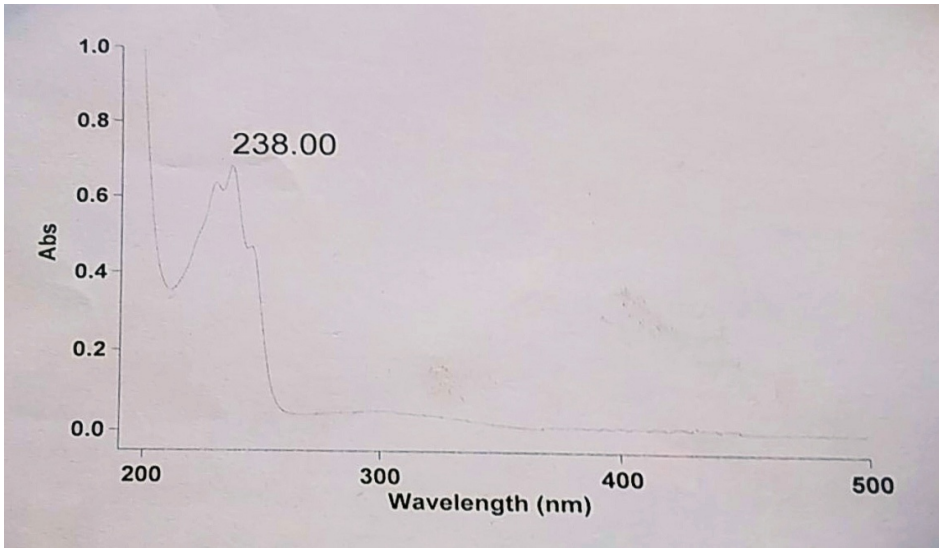


Figure 9.7: Maximum Absorbance of Lovastatin

9.3.4. STANDARD CALIBRATION CURVE OF LOVASTATIN

The ultraviolet spectrophotometric method was used to analyze Lovastatin at wavelength of 238nm. The solutions of Lovastatin in phosphate buffer pH 6.8 were suitably diluted to give concentration ranging from of 2-12 µg/ml. The absorbance was measured at 238 nm and the values are given in the table 9.9.

Table No.9.9: Data for standard curve of Lovastatin in pH 6.8

S.No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.1153±0.002065
3	4	02308±0.005142
4	6	0.3544±0.006825
5	8	0.4786±0.006243
6	10	0.6020±0.007253
7	12	0.7200±0.012972

*Mean ± S.D (n=3)

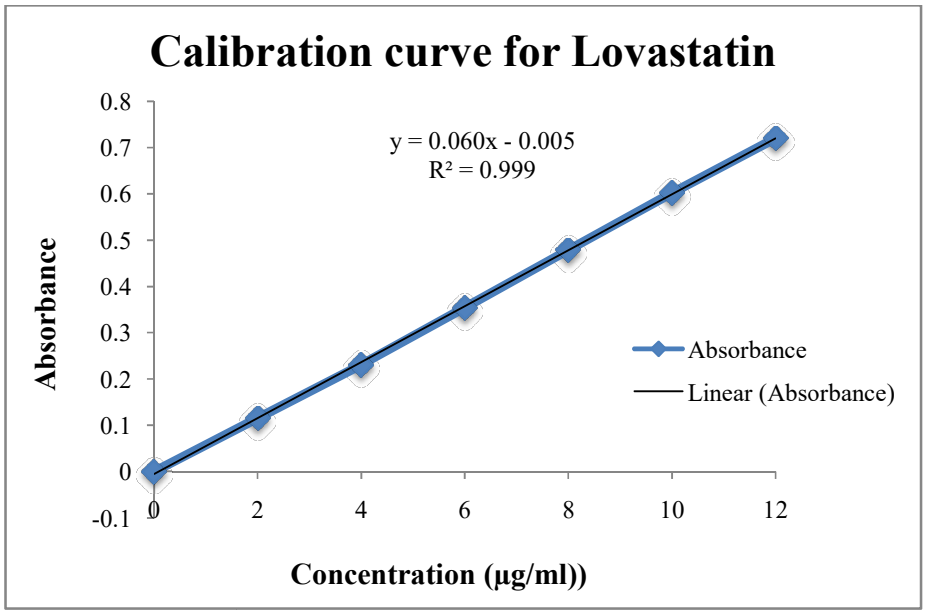


Figure 9.8: Standard graph of Lovastatin in phosphate buffer pH 6.8

Inference

It was found that the solutions show linearity (R^2 = 0.9999) in absorbance at a concentration of 2-12 µg/ml and obeys Beer Lambert's law.

9.3.5. SOLUBILITY STUDIES OF LOVASTATIN

Solubility of Lovastatin pure drug in distilled water and phosphate buffer pH 6.8 was studied and the values are given in the table 9.10.

Table No.9.10: Solubility of Lovastatin pure drug in various media

S.No.	Various Media	Solubility(mg/ml)
1	Distilled water	0.0002116
2	Phosphate buffer pH 6.8.	0.00352055

Inference

Lovastatin pure drug in distilled water and phosphate buffer pH 6.8 was found to be insoluble.

9.4. FORMULATION OF LOVASTATIN LOADED NANOSPONGES

Lovastatin Loaded Nanosponges were prepared by Emulsion solvent evaporation method using Eudragit RS 100 and Ethyl cellulose as Polymers at different ratios (Drug: polymer- 1:1, 1:2, 1:3, 1:4 and 1:5), Polyvinyl alcohol as surfactant and dichloromethane as cross linker as well as solvent.

Figure 9.9: Lovastatin loaded Nanosponges before Lyophilization

Figure 9.10: Lovastatin loaded Nanosponges after Lyophilization

9.5. EVALUATION OF LOVASTATIN LOADED NANOSPONGES

9.5.1. PERCENTAGE YIELD

Table No.9.11: Percentage yield of Lovastatin loaded Nanosponges

Formulation code	Theoretical yield (g)	Practical yield (g)	Percentage yield (%)
F1	1	0.990	99.10
F2	1.5	1.490	99.33
F3	2	1.997	**99.85**
F4	2.5	2.489	98.40
F5	3	2.990	99.66
F6	1	0.975	95.50
F7	1.5	1.450	96.66
F8	2	1.961	**98.05**
F9	2.5	2.490	99.60
F10	3	2.576	85.83

Inference

Lovastatin Loaded Nanosponges were prepared and their percentage yield was calculated. The production yield of all batches was observed in the range of **85.83** to **99.85%.**

It was found that increase in the drug: polymer ratio resulted in increased production yield but after certain concentration it was observed that as the ratio of drug to polymer was increased, the production yield decreased. The results correspond to earlier reports.[46]

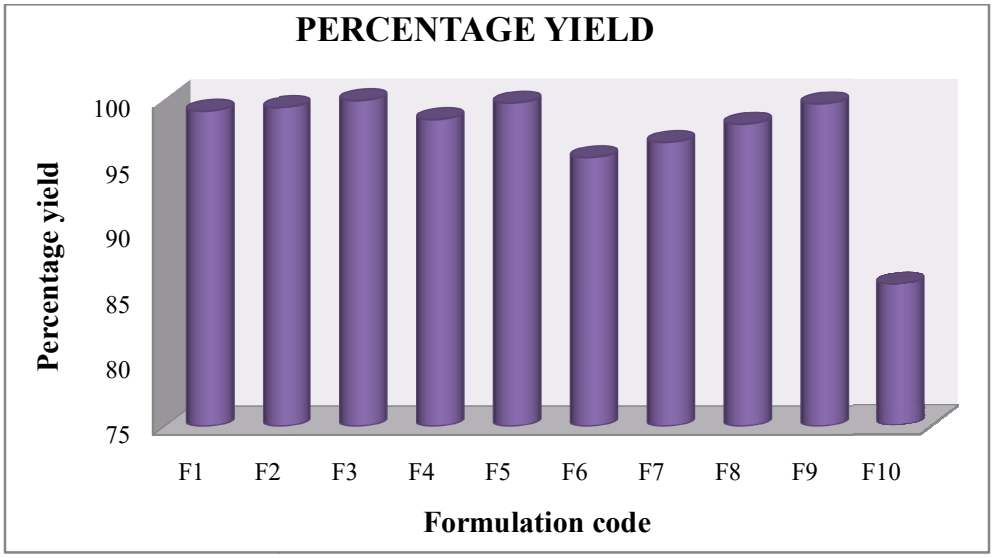


Figure 9.11: **Percentage yield**

9.5.2. DRUG ENTRAPMENT EFFICIENCY

Table No.9.12: Drug Entrapment Efficiency

Formulation code	Entrapment Efficiency (%)
F1	79.48±0.070711
F2	83.73±0.438406
F3	**91.18±0.148492**
F4	90.87±0.735391
F5	82.28±0.438406
F6	81.25±0.438406
F7	83.005±0.289914
F8	**90.04±0.289914**
F9	74.57±1.096016
F10	61.68±0.586899

Mean ±SD (n= 2)

Inference

The entrapment efficiency of the Lovastatin formulations was determined and their entrapment efficiency of the formulations was observed to be between **61.68 %** and **91.18 %.**

The entrapment efficiency of the formulations containing Eudragit RS 100 were found to be in the range of **79.48% to 91.18 %**. The entrapment efficiency of the formulations containing Ethyl cellulose were found to be in the range of **61.68% to 90.04 %.**

The results show that the increase in polymer concentration increased the drug entrapment efficiency. This may be due to higher concentration of the polymer would have provide more space and also reduced escaping of drug into the external phase. But after certain concentration it was observed that the increasing the concentration of the polymer, entrapment efficiency decreased. The results correspond to earlier reports.[51]

The entrapment efficiency was changed when drug and polymer ratio has been changed.

The entrapment efficiency was found to be higher in **F3 (91.18%)** and **F8 (90.04%)** comparatively with other formulations.

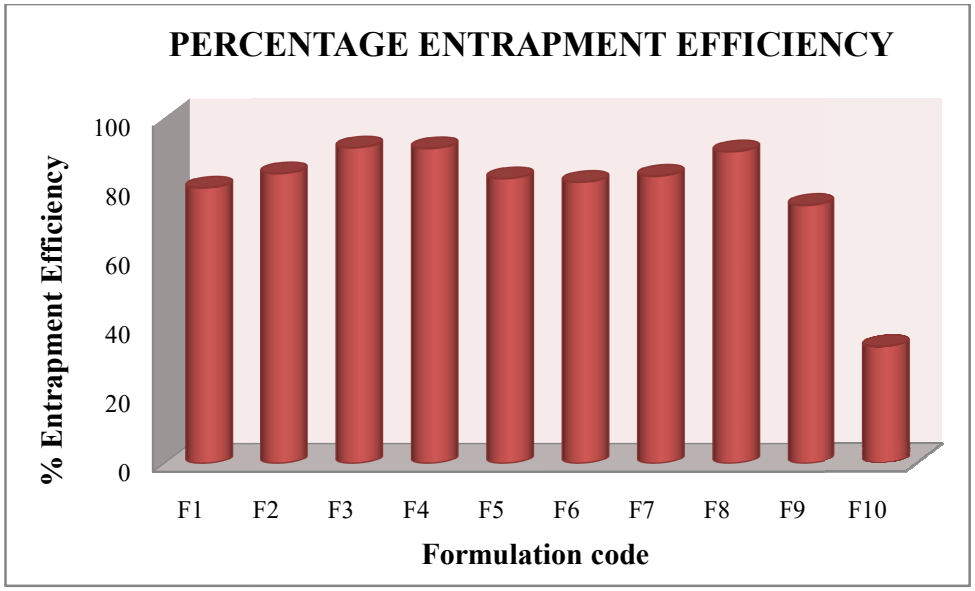


Figure 9.12: **Drug Entrapment Efficiency**

9.5.3. SOLUBILITY STUDIES OF LOVASTATIN LOADED NANOSPONGE

Solubility of Nanosponges in distilled water and phosphate buffer pH 6.8.were studied and the values are given in table 9.13.

Table No.9.13: Solubility of Nanosponges in various media

S.No	Formulation code	Solubility in distilled water (mg/ml)	Solubility in Phosphate buffer pH 6.8 (mg/ml)
1	Pure drug	0.0002116	0.00352
2	F1	1.34125	2.17768
3	F2	1.47379	2.26049
4	F3	1.95408	2.98923
5	F4	1.85472	2.40122
6	F5	1.34125	2.04519
7	F6	1.13427	2.16112
8	F7	1.13827	2.26049
9	F8	1.92925	2.87329
10	F9	1.68082	2.236
11	F10	1.29161	1.95409

Inference

The solubility of all formulations in distilled water and Phosphate buffer pH 6.8 were found to be in the range of **1.29161 to 1.95408 mg/ml** and **1.95409 to 2.98923 mg/ml** respectively.

The solubility of all formulation improved (from insoluble to slightly soluble) compared to pure drug of Lovastatin. Among all the formulations F3 and F8 show higher solubility in distilled water and Phosphate buffer pH 6.8.

Thus the solubility of formulations F3 & F8 in distilled water and Phosphate buffer pH 6.8 were improved (**9234** & **849** fold) and (**9117** & **816** fold) respectively.

9.5.4. *IN VITRO* DRUG RELEASE

In vitro drug release of all the formulation were studied, and percentage of drug release given in table 9.14

Table No.9.14: *In vitro* drug release for all formulations

Time (hrs.)	Cumulative percentage drug release									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
1	20.12	20.01	16.70	7.69	4.72	22.92	19.60	17.41	11.41	7.67
2	36.42	33.54	24.01	18.20	12.24	36.40	31.54	26.13	21.59	13.01
3	44.83	44.90	34.05	26.30	22.30	48.49	46.03	33.51	31.67	21.74
4	60.26	52.18	41.06	35.20	28.47	58.57	50.34	43.10	42.64	27.15
5	67.76	59.46	50.36	41.16	35.55	65.22	64.27	52.27	47.55	36.33
6	74.70	67.33	57.37	45.41	38.84	73.64	72.36	59.76	53.24	41.66
7	87.48	76.47	64.12	51.60	45.39	86.31	82.10	67.19	57.51	47.62
8	95.09	87.31	72.14	58.39	53.45	98.15	92.63	74.08	64.33	55.17
9	-	97.02	78.81	66.80	58.70	-	96.60	80.63	72.03	58.76
10	-	-	88.21	71.17	64.54	-	97.57	87.64	77.86	68.70
11	-	-	93.97	79.19	70.57	-	-	94.54	88.90	73.11
12	-	-	**98.15**	85.74	79.29	-	-	**97.57**	89.55	76.89

Inference

The drug release rate was related to drug: polymer ratio. It was observed that the drug release rate decreased with an increase in the concentration of polymer. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the concentration of polymer in the formulation increases the time required to swell also increases. The results correspond to earlier reports.

In vitro release of optimized formulation showed a rapid initial burst, followed by a very slow drug release. An initial, fast release may be due to more amount of the drug was entrapped near the surface of the Nanosponges due to larger surface area, rather than inside the particles.[58]

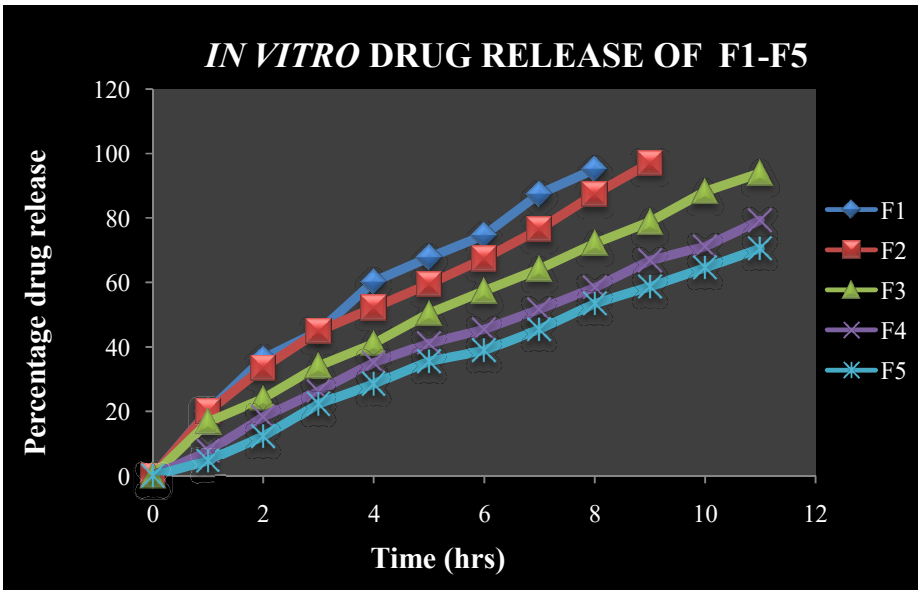


Figure 9.13: ***In vitro*** **release study of Nanosponges (F1-F5)**

Inference

The *in vitro* drug release profile for formulated Lovastatin Nanosponges obtained for F1-F5 formulations are shown in Figure 9.13. Among all the formulations F3 shows controlled release up to 12th hrs Thus F3 was selected as the optimized formulation.

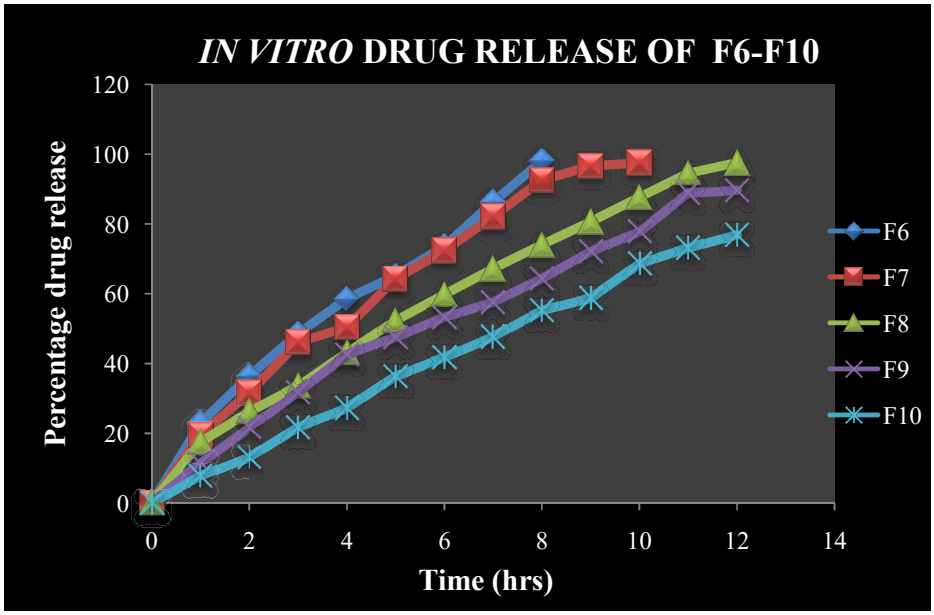


Figure 9.14: ***In vitro*** **release study of Nanosponges (F6-F10)**

Inference

The *in vitro* drug release profile for formulated Lovastatin Nanosponges obtained for F6-F10 formulations are shown in Figure 9.14. Among all the formulations F8 shows controlled release up to 12th hrs. Thus F8 was selected as the optimized formulation.

Based on the entrapment efficiency and *in vitro* drug release F3 and F8 was selected as optimized formulations.

***In vitro* release of optimized formulations and pure drug**

The *in vitro* release data of optimized formulations F3 and F8 are compared with pure drug and the results are given in table 9.15.

Table No.9.15: *In vitro* drug release of Optimized formulations and Pure drug

Time (hrs.)	Cumulative percentage drug release		
	F3	F8	Pure drug
1	16.70	17.41	7.67
2	24.01	26.13	20.24
3	34.05	33.51	50.89
4	41.06	43.10	55.32
5	50.36	52.27	-
6	57.37	59.76	-
7	64.12	67.19	-
8	72.14	74.08	-
9	78.81	80.63	-
10	88.21	87.64	-
11	93.97	94.54	-
12	98.15	97.57	-

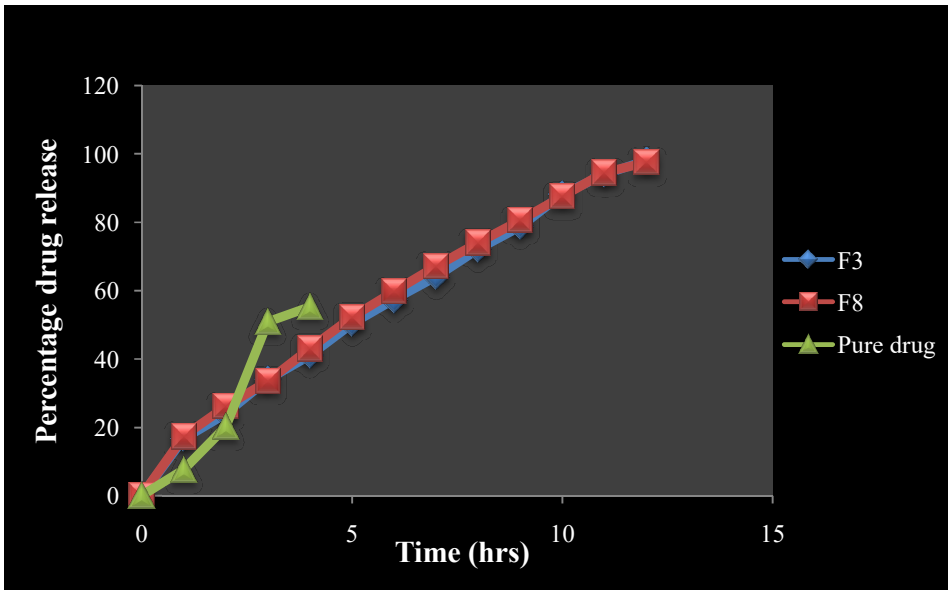


Figure 9.15: ***In vitro*** **release study of optimized formulations and Pure drug**

Inference

The *in vitro* release profile for optimized formulations (F3 & F8) and pure drug of Lovastatin are shown in Figure 9.15. The results of optimized formulations shows (98.15% and 97.57%) controlled release up to 12th hrs, but pure drug shows (50.89%) up to 4th hrs.

These optimized formulations (F3 and F8) characterized for surface morphology, particle size analysis and zeta potential.

9.4.5. SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS

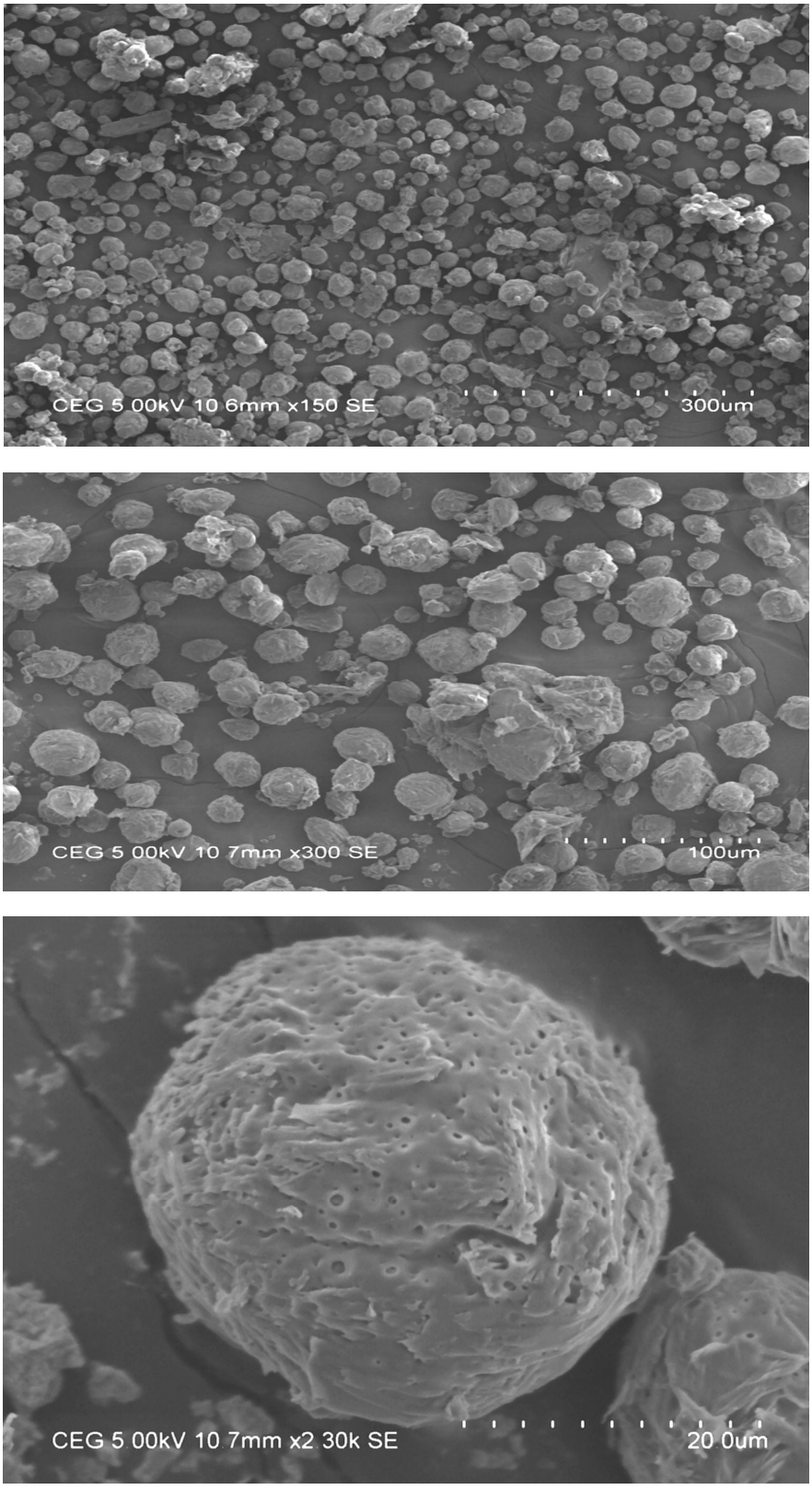
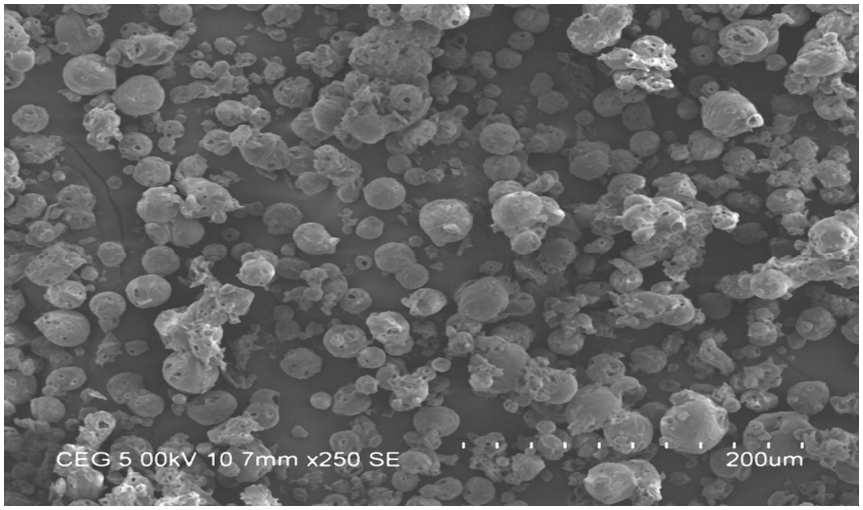
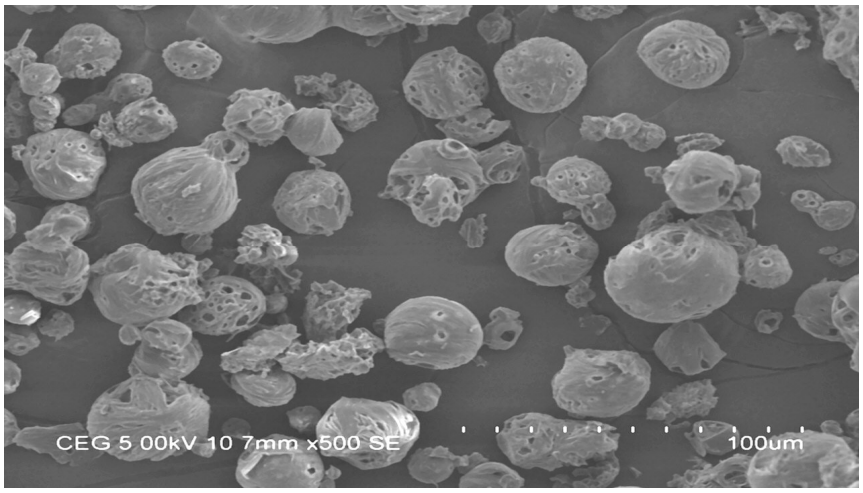


Figure 9.16: SEM image of optimized formulation F3





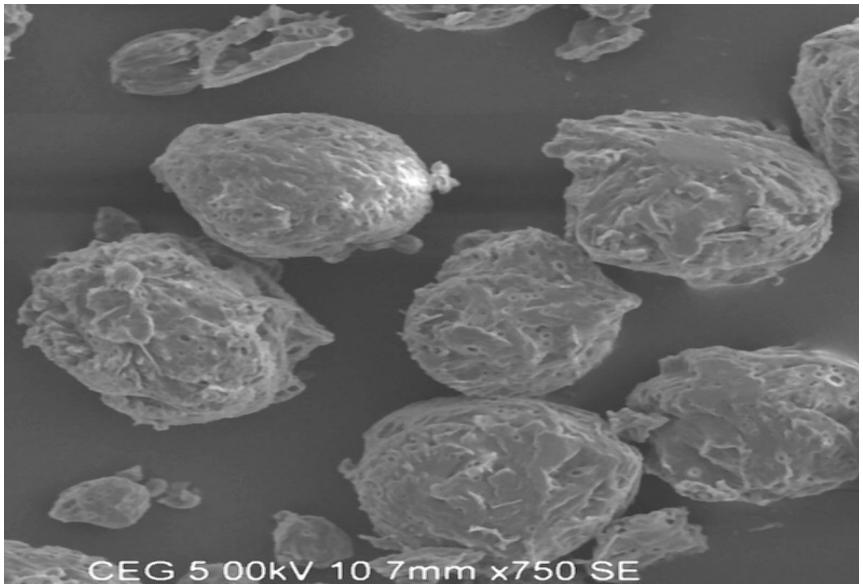


Figure 9.17: SEM image of optimized formulation F8

Inference

The shape and surface morphology of optimized formulations F3 and F8 were observed in scanning electron microscop. It shows that the Nanosponges were spherical with numerous pores on their surface, uniform and spongy in nature. The pores are tunneled inwards which may be due to diffusion of solvent (dichloromethane) from the surface of the Nanosponges.[51]

9.5. 6. DETERMINATION OF PARTICLE SIZE AND POLYDISPERSITY BY MALVERN PARTICLE SIZE ANALYZER

The particle size analysis of optimized F3 formulation was measured. The average particle size is **727.0 nm.** This is within nanometric range (figure 9.18)

The particle size analysis of optimized F8 formulation was measured. The average particle size is **769.5 nm.** This is within nanometric range (figure 9.19)

POLYDISPERSITY BY MALVERN PARTICLE SIZE ANALYZER

PDI indicates the particle size distribution, which ranges from 0 to 1. Theoretically, a monodisperse population indicates PDI equal to zero. The low value of PDI signifies the uniformity of particle size within the formulations.[49]

Inference

Polydispersity of optimized F3 formulation was found to be **0.404** and it shows that uniformity of particle size within formulation.

Polydispersity of optimized F8 formulation was found to be **0.155** and it shows that uniformity of particle size within formulation.

.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	LF3 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	F3.dts	**Dispersant Name:**	Water
Record Number:	7346	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Monday, February 25, 2019 4:...

System

Temperature (°C):	25.0	**Duration Used (s):**	10
Count Rate (kcps):	490.8	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	727.0	**Peak 1:**	768.0	100.0	107.9
PdI:	0.404	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.721	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

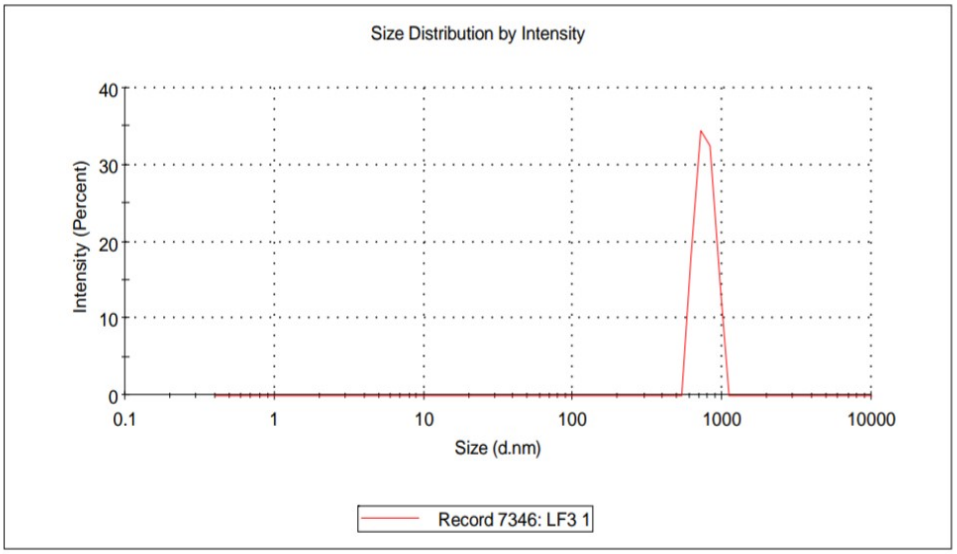


Figure 9.18: Particle size distribution of optimized formulation F3

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	LF8 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	F3.dts	**Dispersant Name:**	Water
Record Number:	7344	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Monday, February 25, 2019 4:...

System

Temperature (°C):	25.0	**Duration Used (s):**	30
Count Rate (kcps):	450.7	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Size (d.nm):	**% Intensity:**	**St Dev (d.n...**
Z-Average (d.nm):	769.5	**Peak 1:**	844.3	100.0	129.3
PdI:	0.155	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.734	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

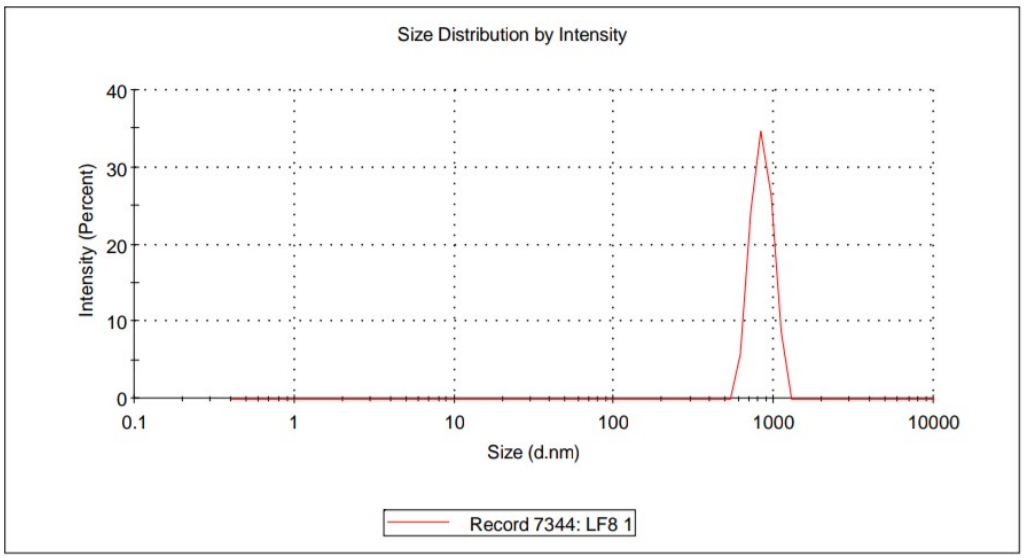


Figure 9.19: Particle size distribution of optimized formulation F8

9.5.7. DETERMINATION OF ZETA POTENTIAL BY ZETA SIZER

The zeta potential for the optimized formulation F3 was found to be **-21.3mV** and it shows that the formulation is stable (figure 9.20).

The zeta potential for the optimized formulation F8 was found to be **-21.6mV** and it shows that the formulation is stable (figure 9.21).

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name:	F3 Z 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	F3.dts	**Dispersant Name:**	Water
Record Number:	7266	**Dispersant RI:**	1.330
Date and Time:	Tuesday, February 19, 2019 1:54...	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	35.3	**Measurement Position (mm):**	4.50
Cell Description:	Zeta dip cell	**Attenuator:**	7

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-21.3	**Peak 1:**	-31.0	61.8	11.0
Zeta Deviation (mV):	16.3	**Peak 2:**	-4.76	38.2	6.77
Conductivity (mS/cm):	0.0234	**Peak 3:**	0.00	0.0	0.00

Result quality : See result quality report

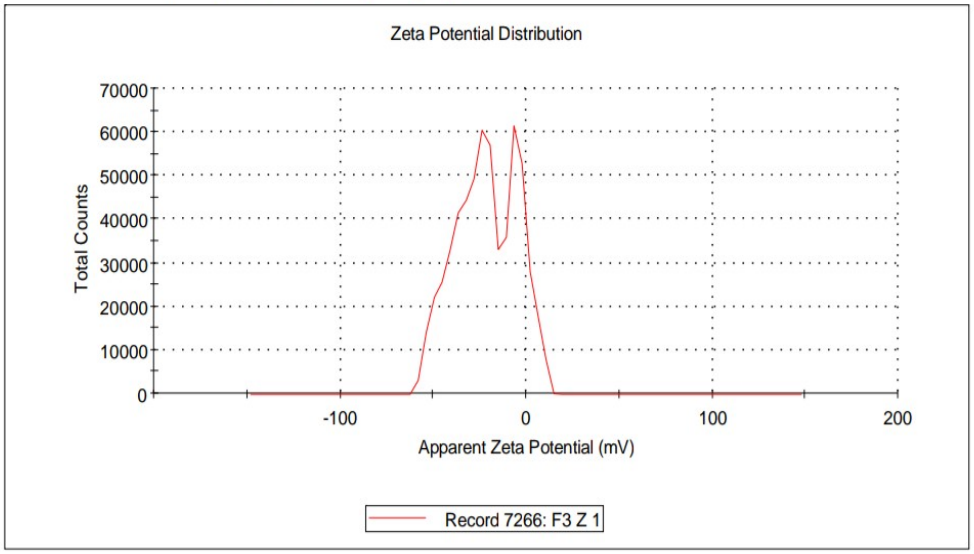


Figure 9.20: Zeta Potential of optimized formulation F3

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name: F8 Z 1

SOP Name: mansettings.nano

General Notes:

File Name:	F3.dts	**Dispersant Name:**	Water
Record Number:	7268	**Dispersant RI:**	1.330
Date and Time:	Tuesday, February 19, 2019 1:58...	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	14
Count Rate (kcps):	205.2	**Measurement Position (mm):**	4.50
Cell Description:	Zeta dip cell	**Attenuator:**	9

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-21.6	**Peak 1:**	-22.7	58.3	10.3
Zeta Deviation (mV):	18.3	**Peak 2:**	-44.4	21.6	4.91
Conductivity (mS/cm):	0.0206	**Peak 3:**	5.44	20.1	5.47
Result quality :	Good				

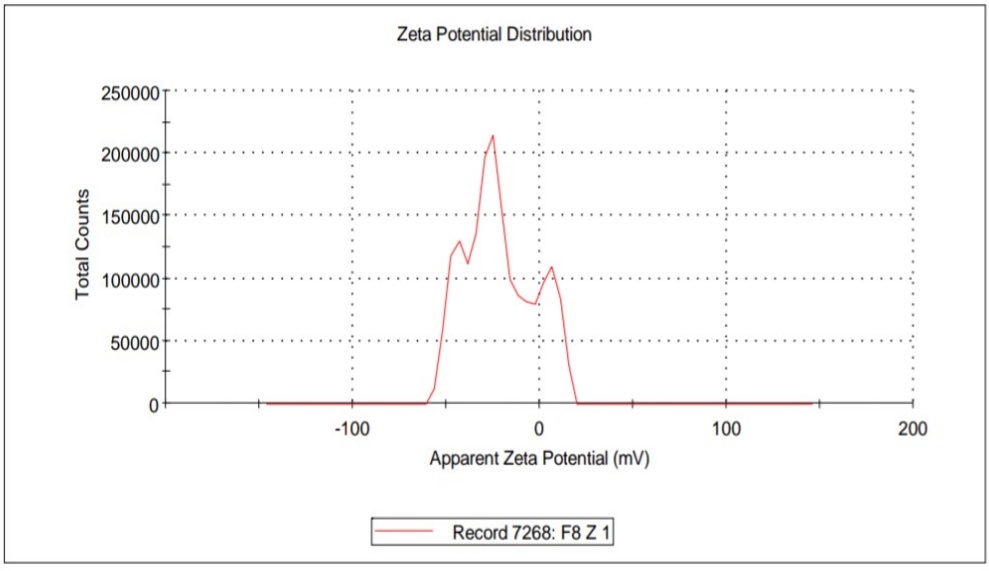


Figure 9.21: Zeta Potential of optimized formulation F3

9.6. PREFORMULATION STUDIES OF OPTIMIZED NANOSPONGES

The optimized formulations F3, F8 and Pure drug were evaluated for flow properties and the results are given in table 9.16

Table No.9.16: Preformulation studies of the pure drug and optimized formulation

F. Code	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner's ratio	Angle of repose (θ)	Porosity (%)
Pure drug	0.306±0.017	0.460±0.038	33.38±1.9	1.501±0.04	48.23±1.26	44.27±0.29
F3	0.274±0.004	0.307±0.010	10.69±1.9	1.119±0.02	28.19±1.48	73.39±0.15
F8	0.161±0.002	0.185±0.003	12.85±1.4	1.147±1.42	30.03±2.90	81.45±0.28

Mean ±SD (n= 3)

Inference

The optimized formulations F3 and F8 have good flow property compared with pure drug have very poor flow.

9.7. OPTIMIZED LOVASTATIN NSs FILLED IN THE CAPSULES

The optimized Nanosponges were filled into "0" size hard gelatin capsules without adding glidant or exicipients because of good flow properties. The filled capsules contains 40 mg of Lovastatin.[98]

Figure 9.22: Lovastatin Nanosponges Filled in Hard Gelatin Capsules

9.8. POST FORMULATION STUDIES FOR CAPSULES

9.8.1. Uniformity of weight

Table No.9.17: Uniformity of Weight for contents in capsules

Formulation Code	Average weight of capsule (g)
C-F3	0.1806±0.001779
C-F8	0.1753±0.000527

Mean ±SD (n=20)

The Capsules comply with the official test for Uniformity of weight.[102]

9.8.2. Disintegration test

Table No.9.18: Disintegration test for Capsules

Formulation code	Time (mins)
F3	8.45
F8	8.33

The Capsules comply with the official test for Disintegration test.[102]

9.8.3. Drug Content

Table No.9.19: Drug Content for Capsules

Formulation code	Drug content (%)
F3	99.32±0.233345
F8	97.64±0.374767

Mean ±SD (n= 2)

The drug content was within the limits (not less than 90% and not more than 110%). It complies with the official standard.[94]

9.9. RELEASE KINETICS OF OPTIMIZED FORMULATIONS

Table No.9.20: Release kinetics of optimized formulation (F3)

Time (hrs)	Cum. % Drug Release	Cum. % Drug remainig	Log Cum. % Drug remaining	Sq. root of time	Log time	Log Cum. % Drug Release	Cube root of % drug Remaining
0	0	100	2	0	∞	∞	4.64158
1	16.70	83.30	1.9206	1	0	1.2227	4.3673
2	24.01	75.99	1.8807	1.4142	0.3010	1.3803	4.2356
3	34.05	65.95	1.8192	1.7320	0.4771	1.5321	4.0402
4	41.06	58.94	1.7704	2	0.6020	1.6134	3.8916
5	50.36	49.64	1.6958	2.2360	0.6989	1.7020	3.6751
6	57.37	42.63	1.6297	2.4494	0.7781	1.7586	3.4933
7	64.12	35.88	1.5548	2.6457	0.8450	1.8069	2.2982
8	72.14	27.86	1.4449	2.8284	0.9030	1.8581	3.0315
9	78.81	21.19	1.3261	3	0.9542	1.8965	2.7672
10	88.21	11.79	1.0715	3.1622	1	1.9455	2.2759
11	93.97	6.03	0.7803	3.3166	1.0413	1.9729	1.8201
12	98.15	1.85	0.2671	3.4641	1.0791	1.9918	1.2276

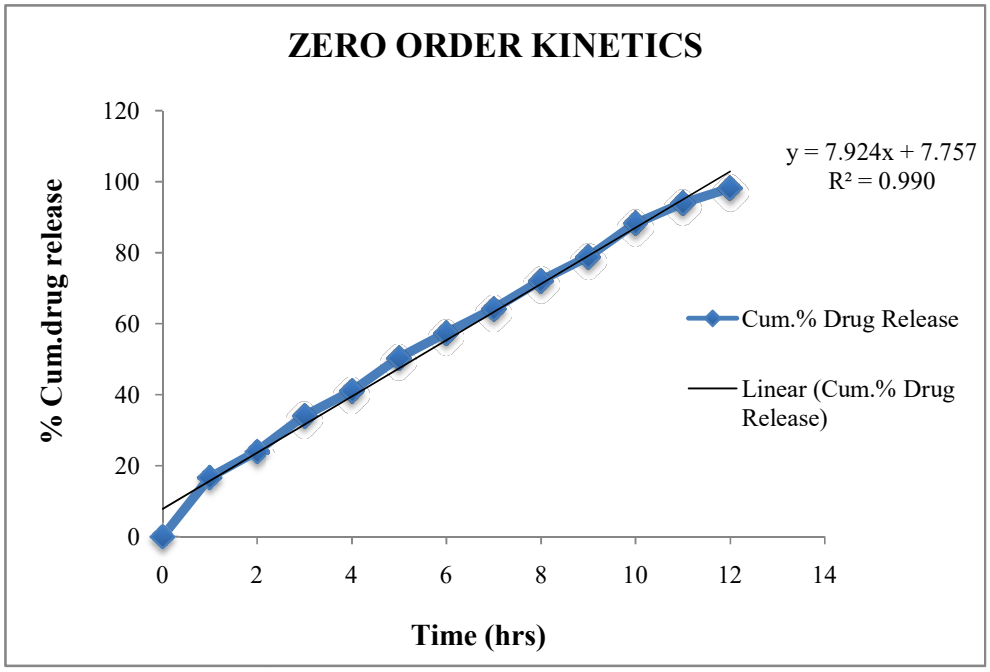


Figure 9.23: A plot of zero order kinetics of optimized formulation (F3)

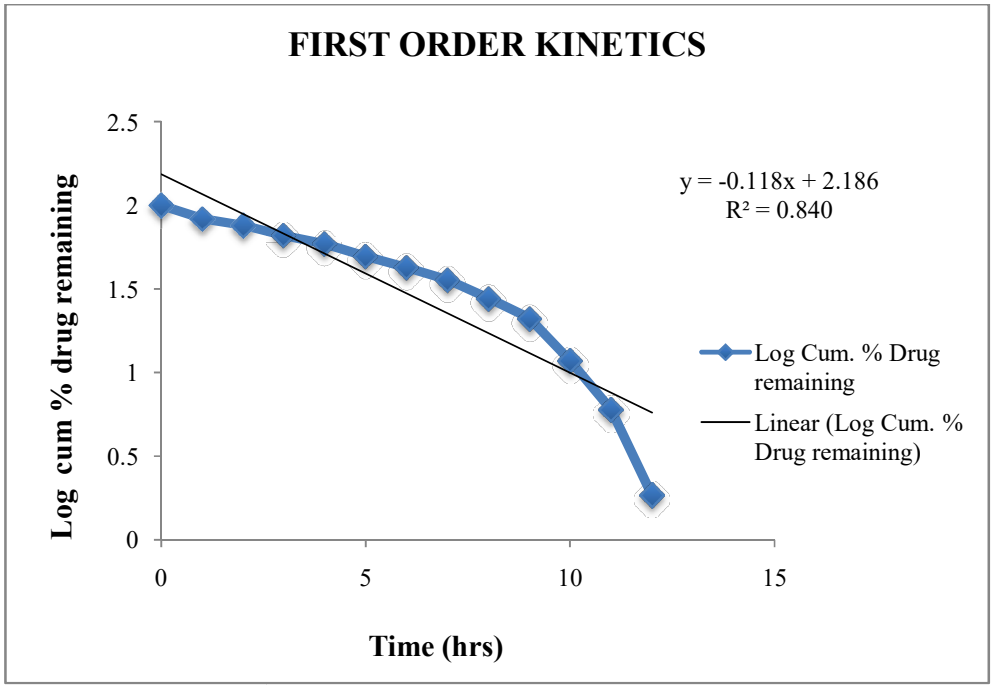


Figure 9.24: A plot of first order kinetics of optimized formulation (F3)

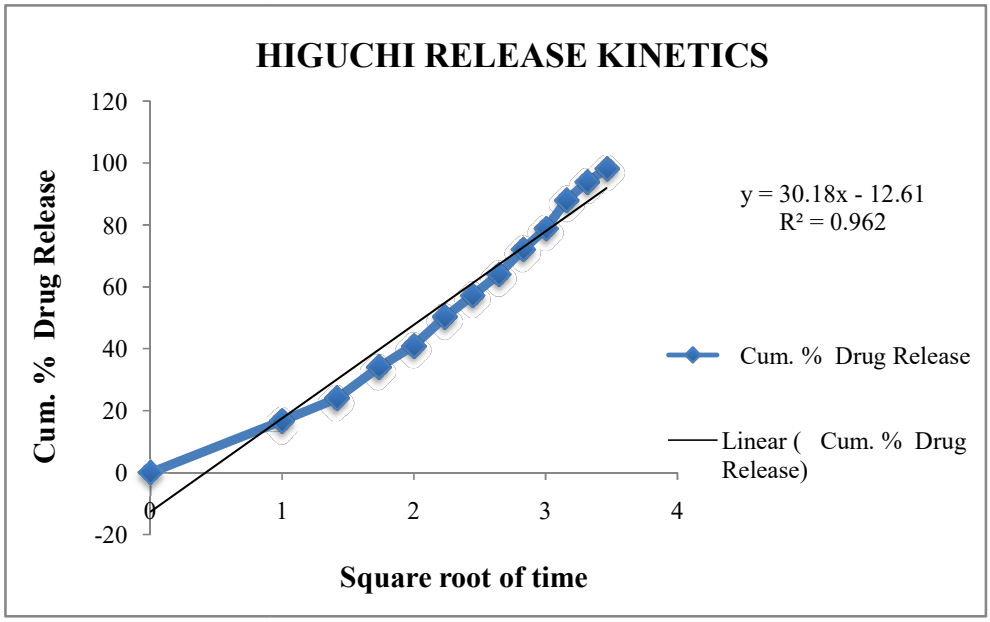


Figure 9.25: A plot of Higuchi release kinetics of optimized formulation (F3)

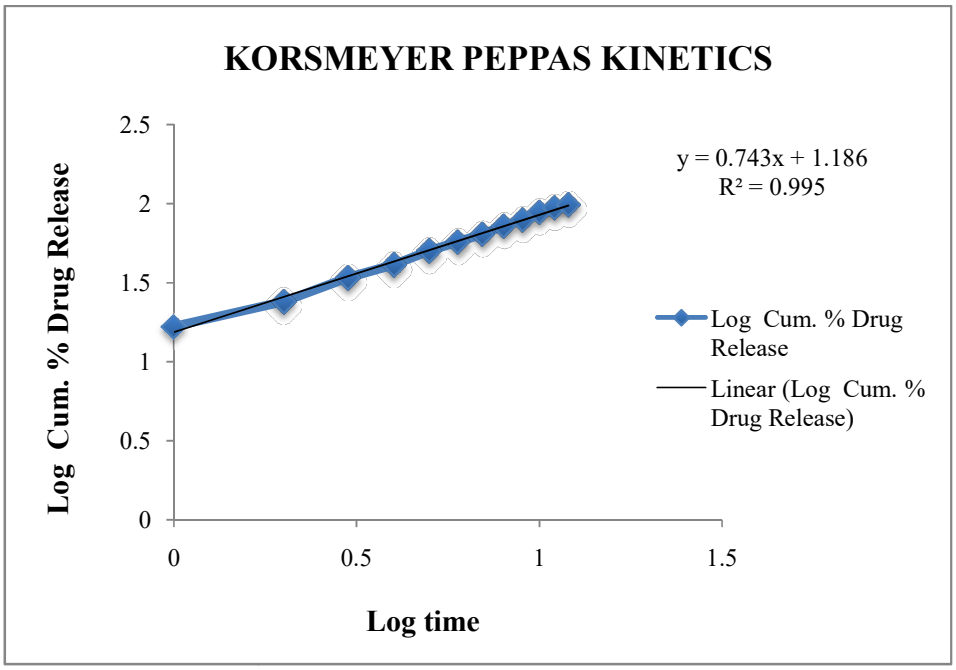


Figure 9.26: A plot of Hixon-Crowell Kinetics of optimized formulation (F3)

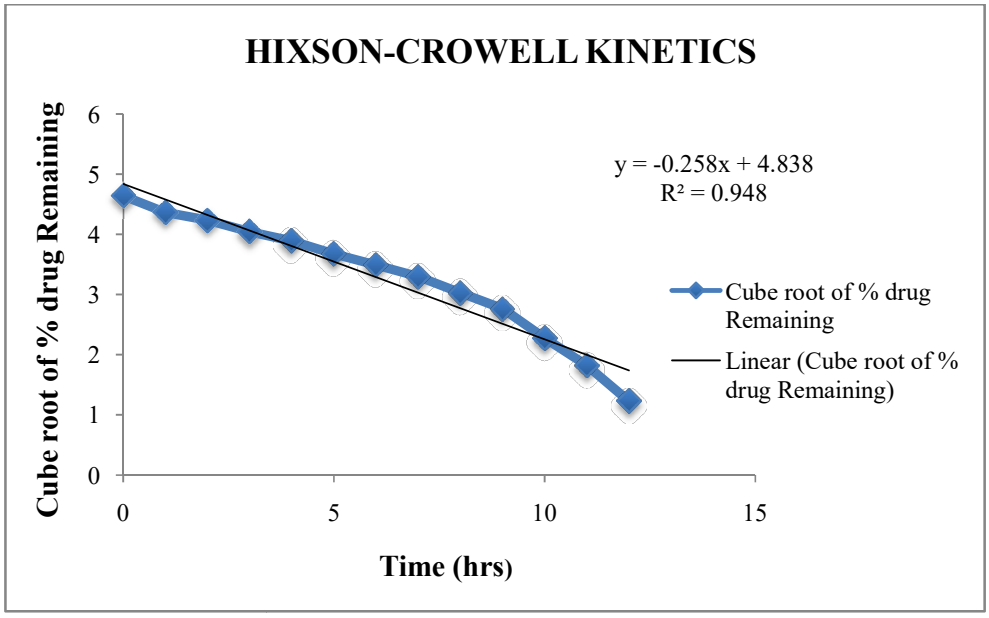


Figure 9.27: A plot of Hixon-Crowell Kinetics of optimized formulation (F3)

Table No.9.21: Release kinetics of optimized formulation (F8)

Time (hrs)	Cum. % Drug Release	Cum. % Drug remaining	Log Cum. % Drug remaining	Sq. root of time	Log time	Log Cum. % Drug Release	Cube root of % drug Remaining
0	0	100	2	0	∞	∞	4.6415
1	17.41	82.59	1.9169	1	0	1.1240	4.3548
2	26.13	73.87	1.8684	1.4142	0.3010	1.4171	4.1958
3	33.51	66.49	1.8227	1.7320	0.4771	1.5251	4.0512
4	43.10	56.9	1.7551	2	0.6020	1.6344	3.8462
5	52.27	47.73	1.6787	2.2360	0.6989	1.7182	3.6274
6	59.76	40.24	1.6046	2.4494	0.7781	1.7764	3.4267
7	67.19	32.81	1.5160	2.6457	0.8450	1.8273	3.2013
8	74.08	25.92	1.4136	2.8284	0.9030	1.8697	3.9594
9	80.63	19.37	1.2871	3	0.9542	1.9064	2.6856
10	87.64	12.36	1.0920	3.1622	1	1.9427	2.3120
11	94.54	5.46	0.7371	3.3166	1.0413	1.9756	1.7660
12	97.57	2.43	0.3856	3.4641	1.0791	1.9893	1.3444

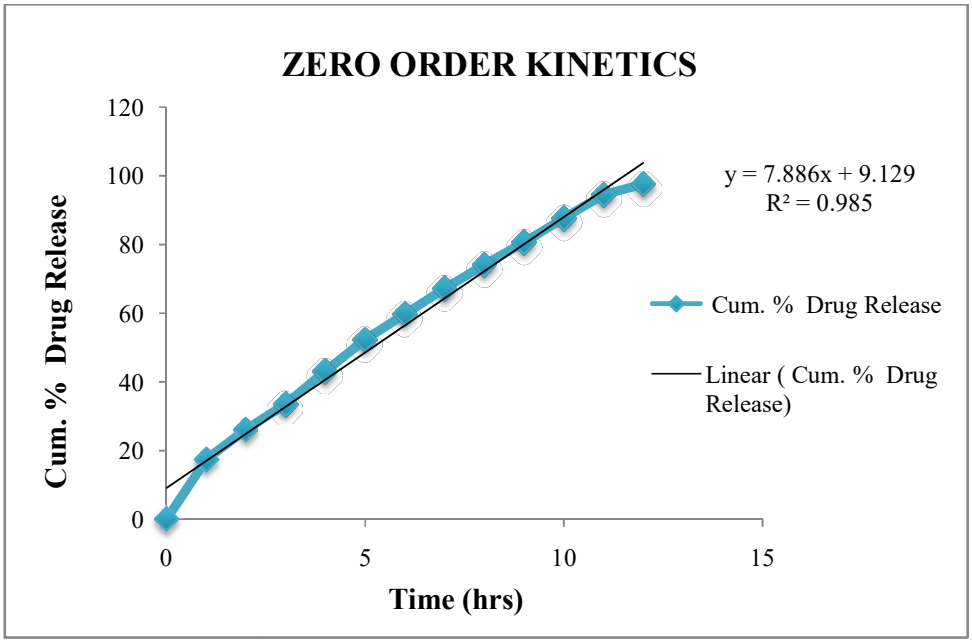


Figure 9.28: A plot of zero order kinetics of optimized formulation (F8)

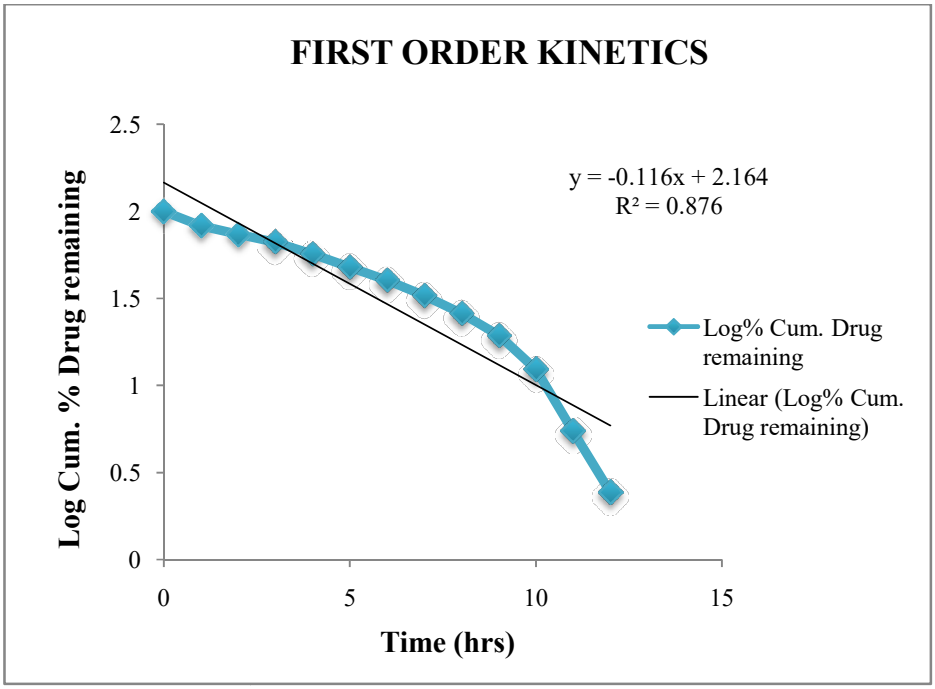


Figure 9.29: A plot of first order kinetics of optimized formulation (F8)

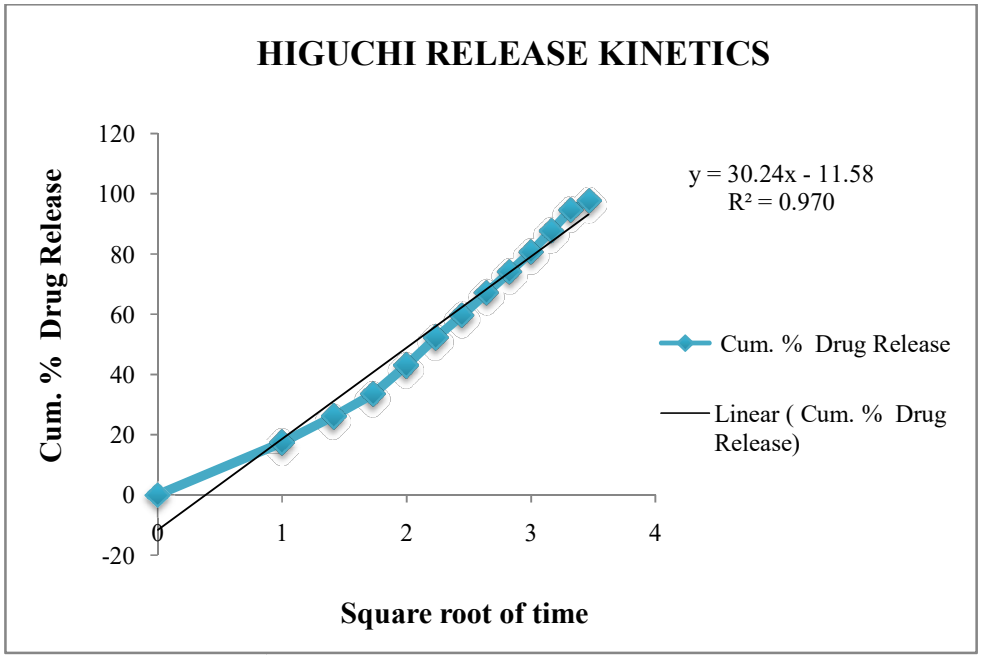


Figure 9.30: A plot of Higuchi release kinetics of optimized formulation (F8)

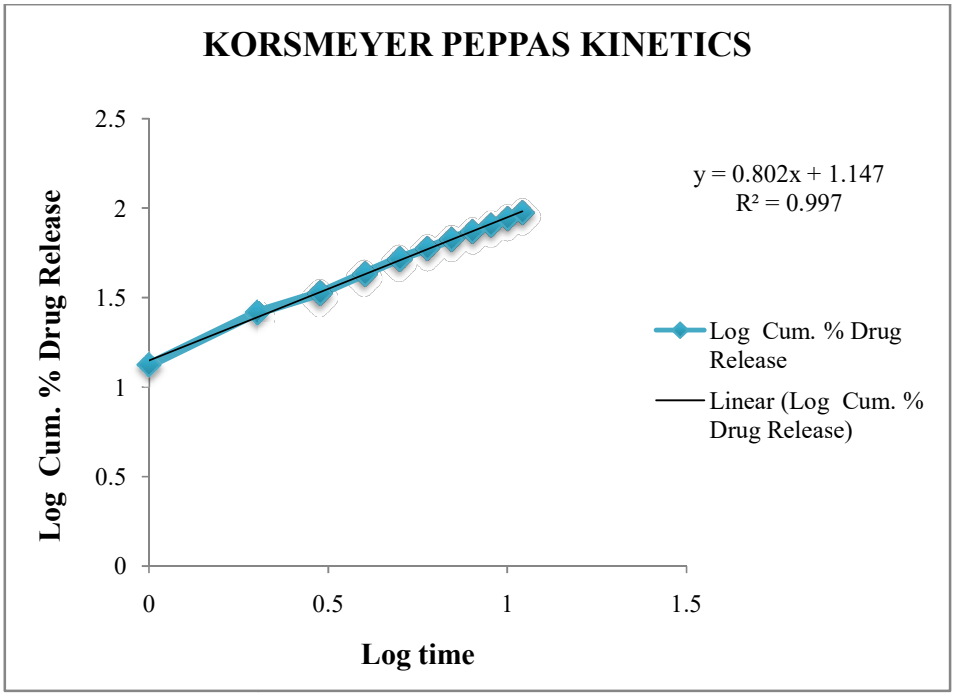


Figure 9.31: A plot of Hixon-Crowell Kinetics of optimized formulation (F8

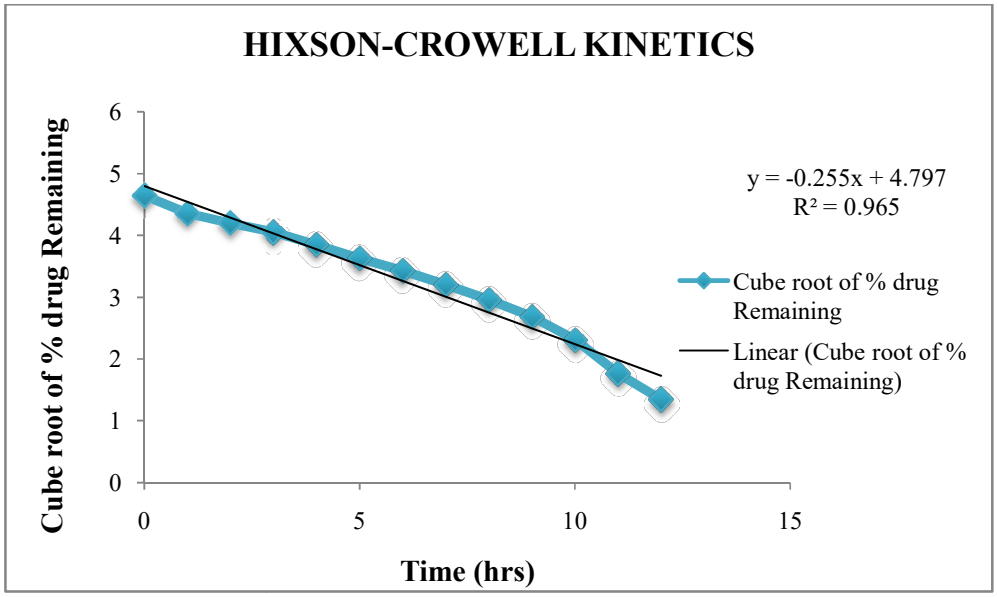


Figure 9.32: A plot of Hixon-Crowell Kinetics of optimized formulation (F8)

The coefficient of determination (R^2) was taken as criteria for choosing the most appropriate model. The R^2values of various models are given in table 9.22.

Table No.9.22: R^2 Values of various Kinetic Models

Kinetic Models	Coefficient of determination (R^2)	
	F3	F8
Zero order	0.9905	0.9853
First order	0.8407	0.8768
Higuchi	0.9628	0.9704
Korsmeyer and Peppas	0.9951	0.9975
Hixson crowell	0.9488	0.9652

- The data from *in vitro* release of optimized formulations F3 and F8 were fit into various kinetic models to find out the mechanism of drug release from Lovastatin Nanosponges.

- A good linearity was observed with the zero order (R^2=00.9905 and 0.9853), the zero order kinetics explains the controlled release of the prepared Nanosponges over the period of 12 hours.
- Higuchi plot (R^2=0.9628 and 0.9704) show linearity, which indicates the rate of drug release through the mode of diffusion and to further confirm the diffusion mechanism, data was fitted into the Korsmeyer Peppas equation which showed linearity.
- The slope of the Korsmeyer Peppas plot (n= 0.743 and 0.802) was found to be more than 0.5 indicating the diffusion was anomalous diffusion (Non Fickian diffusion).
- Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model and showed zero order drug release with anomalous diffusion (Non Fickian diffusion) mechanism.

9.10. STABILITY STUDIES

The optimized formulations (F3 and F8) subjected to stability studies as per ICH guidelines. The results are shown in table 9.23 and table 9.24.

Table No.9.23: Stability data for Optimized Formulation

Stability condition		Physical appearance			Entrapment Efficiency (%)			Drug content (% w/w)		
		Initial	After 45 days	After 90 days	Initial	After 45 days	After 90 days	Initial	After 45 days	After 90 days
40±2°C /75±5 %RH	F3	**NC**	NC	NC	**91.18**	90.67	88.97	**99.32**	99.12	98.43
	F8	**NC**	NC	NC	**90.04**	89.63	88.08	**97.64**	97.31	95.97
4±2°C	F3	**NC**	NC	NC	**91.18**	91.10	90.21	**99.32**	99.31	98.42
	F8	**NC**	NC	NC	**90.04**	89.94	89.18	**97.64**	97.34	96.14

*NC-No change

Table No.9.24: Stability data for Optimized Formulation (Cumulative % drug release of Optimized formulation)

Time (hrs)	Cumulative % drug release									
	40±2°C/75±5%RH						4±2°C			
	F3			F8			F3		F8	
	Initial	After 45 days	After 90 days	Initial	After 45 days	After 90 days	After 45 days	After 90 days	After 45 days	After 90 days
1	**16.70**	15.26	14.98	**17.41**	16.21	16.05	16.12	15.75	17.22	16.66
2	**24.01**	24.04	23.70	**26.13**	25.78	24.90	23.69	22.92	26.16	25.75
3	**34.05**	32.97	32.29	**33.51**	33.11	32.13	34.13	33.53	32.96	32.15
4	**41.06**	40.27	40.05	**43.10**	41.83	41.25	42.11	41.50	42.77	43.81
5	**50.36**	50.06	49.62	**52.27**	51.73	51.60	51.29	49.83	51.96	51.07
6	**57.37**	55.93	56.12	**59.76**	59.05	58.34	56.75	58.07	59.08	58.76
7	**64.12**	62.94	62.31	**67.19**	65.84	65.12	65.07	63.54	66.98	66.91
8	**72.14**	71.11	70.93	**74.08**	72.93	72.09	71.15	70.74	74.22	73.66
9	**78.81**	77.36	76.96	**80.63**	79.26	78.62	78.69	79.09	81.10	78.73
10	**88.21**	86.93	86.77	**87.64**	86.73	86.16	87.72	89.04	88.22	86.34
11	**93.97**	92.21	91.97	**94.54**	92.41	92.11	93.08	92.90	93.45	93.27
12	**98.15**	97.25	96.05	**97.57**	96.09	95.09	98.07	97.05	97.11	96.16

Inference

No significant changes in Physical appearance, entrapment efficiency, drug content and *in vitro* drug release at storage condition of 40°C ± 2°C / 75 ± 5% RH and 4±2°C after the end of 0, 45 days and 90 days.

SUMMARY

&

CONCLUSION

10. SUMMARY AND CONCLUSION

The purpose of this research was to prepare Lovastatin Nanosponges for controlled release of drug, to improve the solubility, reduce dose dependent side effects and improve the patient compliance.

Lovastatin is a poorly soluble drug with a short half life, thus selected as a model drug for Nanosonges Drug Delivery System and to release the drug in a controlled manner for prolonged period. Lovastatin is formulated as Nanosponges by Emulsion Solvent Evaporation method using Eudragit RS 100 and Ethyl Cellulose as a Polymers, Polyvinyl alcohol as a stabilizer and finally enclosed in hard gelatin Capsules.

- The Physical compatibility of Lovastatin with excipients were studied. The drug and exicipients were physically compatible with each other.
- The chemical compatibility studies of Lovastatin with excipients were carried out using FTIR Spectrometer. It revealed no interaction between the drug and excipients.
- Melting point of drug was determined. It matches with standard value.
- Calibration curve was plotted for Lovastatin and it was found that the solutions show linearity (0.999) and obeyed Beer's and Lambert's law.
- Solubility of pure drug was determined. Lovastatin pure drug in distilled water and phosphate buffer pH 6.8 was found to be insoluble.
- Lovastatin loaded Nanosponge formulations were prepared by Emulsion Solvent Evaporation method using Eudragit RS 100 and Ethyl Cellulose as Polymers at different ratios (Drug: polymer- 1:1, 1:2, 1:3, 1:4 and 1:5). The Polyvinyl alcohol was used as surfactant, dichloromethane as cross linker as well as solvent.
- All formulations were evaluated for percentage yield was to be in the range of 85.83 to 99.85%, which is in increasing order due to increases in the concentration of polymer.
- The entrapment efficiency of all the formulations were found to be in the range of 61.68 to 91.18%. The results show that the increase in polymer concentration

increased the drug entrapment efficiency but after certain concentration, increasing the concentration of the polymer, entrapment efficiency decreased. The entrapment efficiency was found to be higher in F3 (90.04%) and F8 (91.18%) comparatively with other formulations.

- The solubility of all formulation in distilled water and Phosphate buffer pH 6.8 were found to be in the range of 1.29161 to 1.95408 mg/ml and 1.95409 to 2.98923 mg/ml. The solubility of all formulation improved (from insoluble to slightly soluble) compared to pure drug of Lovastatin.

- The *in vitro* release was carried out for all the formulations. These results showed that as the concentration of polymer was increased, the drug release rate was decreased. The formulation F3 (containing 1:3 ratio of LOV and Eudragit RS 100) released 98.15% and F8 (containing 1:3 ratio of LOV and Ethyl cellulose) released 97.57% at the end of 12th hour. The release profile was in controlled manner comparatively with other formulations.

- Based on the higher entrapment efficiency and prolonged *in vitro* drug release F3 and F8 were selected as optimized formulations.

- The optimized formulation F3 and F8 were characterized for surface morphology, particle size analysis and zeta potential.

- The shape and surface morphology of optimized formulations were observed in SEM. It shows that the Nanosponges were spherical with numerous pores on their surface, uniform and spongy in nature. The presence of pores may be due to the diffusion of the solvent (dichloromethane).

- The particle size distribution and polydispersity done by Malvern particle size analyzer. The average particle size of formulation F3 was 727.0 nm and F8 was 769.5 nm and within nanometric range.

- Polydispersity of formulation F3 and F8 were found to be 0.404 and 0.155 respectively, it shows that uniformity of particle size within formulation.

- The zeta potential study was done by Malvern zeta sizer. The zeta potential for the optimized formulations F3 and F8 were found to be be -21.3mV and -21.6mV respectively, it shows that the formulation is stable.

- Flow property measurements (Bulk density, Tapped density, Angle of repose, Carr's index and Hausner's ratio) and Porosity were carried out for Lovastatin pure drug and optimized Nanosponges. It revealed that the flow property of pure drug was very poor, but the Lovastatin Naosponges have good flow.

- The optimized Nanosponge formulation F3 and F8 were filled into "0" size hard gelatin capsules without adding glidant because of its good flow property.

- Post formulation parameters (uniformity of weight, disintegration test, drug content, and *in vitro* drug release) of capsule were evaluated. The results were found to comply with official specifications.

- The release kinetics of the optimized formulations were fitted to various kinetic models and the formulations F3 and F8 were best fitted to Zero order kinetics. The zero order kinetics explains the controlled release of the prepared Nanosponges over the period of 12 hours.

- Higuchi plot show linearity, which indicates the rate of drug release through the mode of diffusion and the slope of the Korsmeyer Peppas plot indicating the diffusion was anomalous diffusion (Non Fickian diffusion).

- Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model and showed zero order drug release with Non Fickian diffusion mechanism.

- The optimized formulations F3 and F8 were subjected to accelerated stability study (temperature 40°C±2°C and RH 75±5%) and refrigerator temperature (4 ± 2^0C). The results show no significant change in appearance, dug entrapment efficiency, drug content and *in vitro* dissolution profile of optimized formulations after 3 months.

- The foregoing results attempt to suggest that for highly lipophilic drugs like Lovastatin, Nanosponge approach would be a possible alternative delivery system to conventional oral formulation to improve its bioavailability.

Future scope

1. *In-vivo* study
2. To study the pharmacokinetic and bio distribution of the drugs.

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ANNEXURE

Om Sakthi

ADHIPARASAKTHI COLLEGE OF PHARMACY

MELMARUVATHUR - 603 319, TAMILNADU.
Tel: 044/27529093; Fax: 044-27529196

(Accredited by 'NAAC' with a CGPA of 2.80 on a Seven point scale at "B++" Grade)

CPE Programme on

"CURRENT TRENDS IN BRAIN TARGETED DRUG DELIVERY SYSTEMS FOR NEURO DEGENERATIVE DISORDERS"

Co-Sponsored By

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

Date: 05.01.2018

CERTIFICATE OF PARTICIPATION

This is to certify that ~~Dr./Mr./Mrs./~~Ms .Ranjitha. R..... of College of Pharmacy, Madras Medical College.

Participated as Delegate/~~Resource Person~~ in the CPE Programme held on 05.01.2018 at Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamilnadu.

S. Shanmugam

Dr.S. Shanmugam, M.Pharm., Ph.D.
Organizing Secretary

Dr. T. Vetrichelvan, M.Pharm., Ph.D.
Convener/ Principal

Dr. E. Srilekha Senthilkumar, M.B.B.S., D.G.O.
Correspondent

DEPARTMENT OF PHARMACOLOGY
Govt. Kilpauk Medical College &
The T.N.Dr.M.G.R. Medical University, Guindy, Chennai.

"PHARMKINETIKOS"

Certificate of Participation

This is to certify that.....Mrs. Ranjitha. R..... has participated as Delegate/Speaker in the CME on " PHARMACOKINETICS" conducted by the Department of Pharmacology, Govt. Kilpauk Medical College, Chennai on 17th August, 2018.

This CME is accredited within 10 credit points under Category II by The TN.Dr.M.G.R Medical University, Guindy, Chennai.

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Certificate

This is to certify that

Prof./Dr./Mr./Ms. RANJITHA.R

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	Dr. Shailendra Saraf	Dr. Dhirender Kaushik	Dr. Ashish Baldi
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

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P.G Pharmacy - Research Fellowship Award

This is to certify that Ms. / Mr. Ranjitha. R

student of College of Pharmacy, Madras Medical College, Chennai

Faculty of Pharmacy, Pharmaceutics *Department, has been*

awarded scholarship of Rs 10,000/- *for securing* Second *rank*

in the M.Pharm Project for the academic year 2018 - 2019

N. SREENIVASEN
Hon. Gen. Secretary

Date : 04-01-2019

S.V. VEERRAMANI
Chairman

DEPARTMENT OF PHARMACOLOGY,
GOVT.KILPAUK MEDICAL COLLEGE &
The TN.Dr.M.G.R. MEDICAL UNIVERSITY, GUINDY, Chennai.

CME on "BIOAVAILABILITY - BIOEQUIVALENCE STUDIES"

Certificate of Participation

This is to certify that R. Ranjitha *has participated as Delegate/Speaker in the CME on "BIOAVAILABILITY - BIOEQUIVALENCE STUDIES" conducted by the Department of Pharmacology, Govt. Kilpauk Medical College. Chennai in association with CDSCO, Chennai on 1st September,2017.*

This CME is accredited with 10 credit points under Category II by The TN.Dr.M.G.R. Medical University, Guindy, Chennai.

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