FORMULATION DEVELOPMENT AND CHARACTERIZATION OF SIMVASTATIN LOADED LONG ACTING MICROSPHERES

A Dissertation Submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI- 600 032

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

MASTER OF PHARMACY
IN
BRANCH-I >> PHARMACEUTICS

Submitted by,
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Under the guidance of
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KOMARAPALAYAM -638 183
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APRIL-2020

Certificates

EVALUATION CERTIFICATE

This is to authenticate that the dissertation work entitled **" FORMULATION DEVELOPMENT AND CHARACTERIZATION OF SIMVASTATIN LOADED LONG ACTING MICROSPHERES "**, submitted by the Student bearing **Reg.no: 261810262** to **" The Tamil Nadu Dr. M.G.R. Medical university -Chennai "**, in partial fulfilment for the award of Degree of **Master of Pharmacy in Pharmaceutics** was evaluated by us during the examination held on ……...

Internal Examiner **External Examiner**

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DECLARATION

I SENTHAMIL SELVAN T , herewith declare that the dissertation entitled "**FORMULATION DEVELOPMENT AND CHARACTERIZATION OF SIMVASTATIN LOADED LONG ACTING MICROSPHERES",** submitted to "The Tamil Nadu Dr.M.G.R. Medical university-Chennai", in partial fulfillment and requirement of university rules and regulation for the award of Degree of Master of Pharmacy in Pharmaceutics, is a bonafide research work has been carried out by me during the academic year2019-2020, under the guidance and supervision of Ms.MANODHINI ELAKIYA M.Pharm., Assistant professor, Department of Pharmaceutics, J.K.K. Nattraja college of pharmacy, Komarapalayam.

I additionally declare that this research work is genuine, and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is original to the best of my knowledge.

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SENTHAMIL SELVAN T
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Dedicated to god almighty ,

My Strong pillars (Father and Mother)

Origin of my strength (Sisters & Friends)

Source of my knowledge (Guide and guru)

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ABBREVIATIONS

Abbreviation	Full form stands
IID	Inactive ingredient database
($t_{1/2}$)	Biological half-life
°F	Fahrenheit
API	Active pharmaceutical ingredient
AUC	Area under curve
BCS	Biopharmaceutical classification system
CR	Controlled release
CRDDS	Controlled release drug delivery system
DDS	Drug delivery system
DPI	Dry powder inhaler
DSc	Differential scanning calorimetry
ER	Extended release
FTIR	Fourier transform infrared spectroscopy
HMG-CoA	Hydroxy-3-methylglutaryl-coenzyme A
HPMC	Hydroxy propyl methyl cellulose
ICH	International Council for Harmonization
IDD-P	Insoluble Drug Delivery Microparticle
mL	Milliliter
NLC	Nano structured lipid carriers
nm	Nanometer
NO	Nitric oxide
O/W	oil-in-water
PDGF	platelet-derived growth factor
Pdi	polydispersity index
PEG	polyethylene glycol
PGA	Poly(glycolides)

PLGA	Poly(lactide-coglycolides)
pMDI	Pressurized metered-dose inhaler
PSD	Particle size distribution
PVA	Poly vinyl alcohol
PVP	Polyvinyl pyrrolidine
RH	Relative humidity
RT	Room temperature
S.c	Subcutaneous
SAA	Supercritical assisted atomization
SDP	spray dried solid dispersion
SEDDS	Self Emulsifying Drug Delivery System
SEM	Scanning Electron microscopy
SLS	Sodium lauryl sulfate.
SMV	Simvastatin
SMVF	Simvastatin formulation
SNEs	self-Nano emulsifying systems
SVA	Simvastatin hydroxy acid
tPA	tissue Plasminogen Activator
Vd	volume of distribution
W/v	Weight by volume
w/w	Weight by weight
μm	Micrometer

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NOVEL DRUG DELIVERY SYSTEM

1.0 INTRODUCTION TO NDDS

*N*ovel Drug delivery System (NDDS) refers to the perspective of formulations, technology, and systems for delivering a pharmaceutical compound in the body as required to attain its desired therapeutic effects. NDDS is a combination of advance technique and new dosage forms which are far better than conventional dosage forms.

CONTROLLED RELEASE DDS

The term of controlled release drug delivery system, which exhibits a system that provides a control on drug release pattern in the biological system. This system assures to control the concentration of drug to the target area and maintains the desired drug level within the body. Controlled release DDS are employed to achieve therapeutic goals with any drug therapy, the delivery system or dosage regimen and it should be capable to attain the therapeutic plasma levels immediately and maintenance of drug concentration levels for the entire duration of therapy. Controlled drug release generally obtained "zero-order" release from the dosage form. Zero-order release comprises drug release from the dosage form, which is independent of the amount of drug in the delivery system . [1,2]

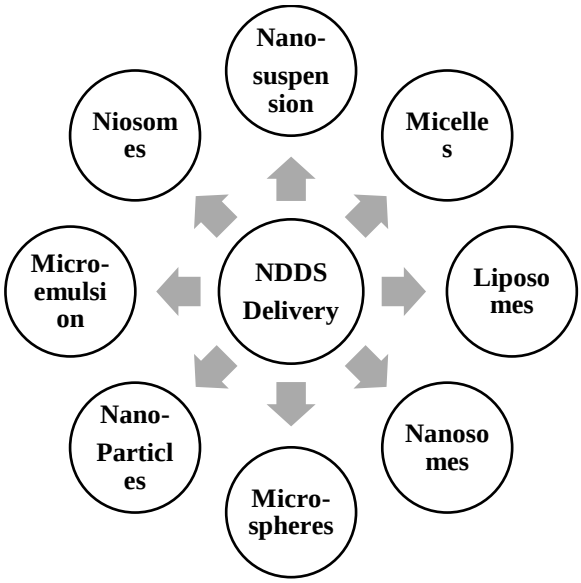


Figure 01: Pictorial representation of NDDS

MICROSPHERES

1.1 Introduction to Microspheres

Microspheres are novel drug delivery formulations containing small spherical particles with diameters ranges from 1 to 1000 μm. On another hand the microspheres are widely known as microparticles or microparticulate system. Microspheres can be formulated from several polymeric materials which is originate from natural, semi-synthetic and synthetic materials or even from inorganic materials. The methods of microsphere production are varying offers an innumerable of opportunities to control the aspects of administration of the pharmaceutical compound. Microparticulate drug delivery focus to facilitate the precise release of the expected amount of a drug component at the site of action and its entry minimizes at nontarget sites.

The exploitation of these changes in pharmacokinetic behavior can lead to an improved therapeutic effect. The objective of any pharmaceutical drug administration system is to provide a therapeutic amount of the compound at the target site in the body to rapidly achieve an effective concentration and maintain the dose for given time. A well-designed controlled release system for the compound can overcome some of the problems of conventional therapy and improve the therapeutic efficacy. [3]

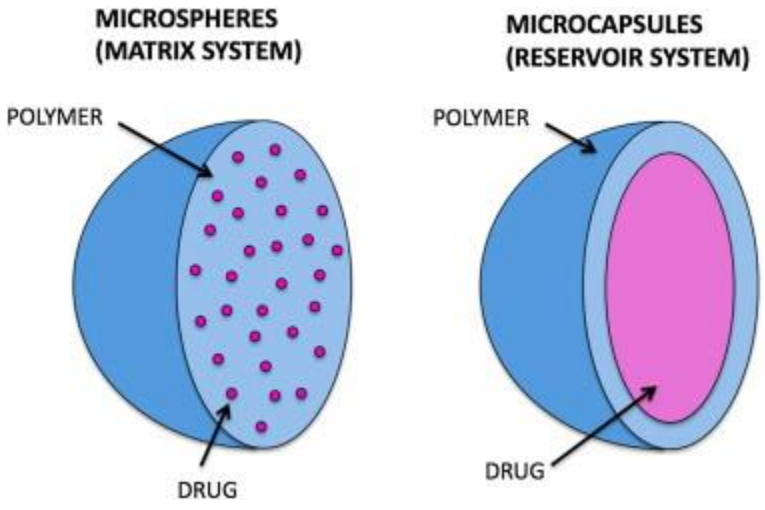


Figure 02: Pictorial representation of microsphere

1.2 Merits of Microsphere DDS

- Reduction in size leads to increase in surface area which can enhance solubility of the poorly soluble drug.
- Decrease dose and toxicity.
- Less dosing frequency leads to better patient compliance.
- Provide constant drug concentration in blood which can increase patent compliance,
- Coating of drug with polymers helps the drug from enzymatic degradation and suitable for delivery.
- Better drug utilization will improve the bioavailability and reduce the incidence of adverse effects.
- Protects the GIT from irritant effects of the drug.
- Reduce the reactivity of the core in relation to the outside environment.
- Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.
- Convert liquid to solid form and to mask the bitter taste.
- Extended release delivery of biodegradable microspheres is used to control drug release rates and eliminating the inconvenience of repeated injections. [4]

1.3 Limitations

- Release rate can differ from one dose to another dose.
- Changing in process variables like change in temperature, evaporation, pH, solvent addition, and /agitation may influence the stability of core particles to be encapsulated.
- Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity
- The costs of the materials are substantially higher than those of standard formulations.
- The fate of polymer matrix and its effect on the environment. [5]

1.3.0 METHOD OF PREPARATION OF MICROSPHERES

The rate-controlled microspheres are fabricated via some techniques lab-scale as well as industrial commercial scale. The choice of technique depends upon the nature of polymer as well nature of drug and the duration of therapeutic needs. Generally, the microsphere formulation is prepared by the methods are explained below:- [6,7,8,9,10]

S.R.No	Method of preparation
1	Single emulsion technique
2	Multiple emulsion techniques
3	Coacervation/Phase Separation
4	Polymerization Technique
5	Spray Drying
6	Solvent extraction
7	Solvent Exchange method
8	Microfluidic Flow-Focusing Method
9	Supercritical Assisted Atomization

1.3.1 Single-Emulsion Solvent Evaporation

In single emulsion technique under solvent evaporation the systems are broadly classed into two major system as Oil-in-Water (O/W) and Water-in-Oil (W/O).In solvent evaporation method is particularly suitable for micro-encapsulation of lipophilic drugs (either dispersed or dissolved) in the dispersed phase of a volatile solvent. Natural polymers are dissolved in aqueous medium and the dispersion phase in non-aqueous medium (oil). Further, crosslinking of the dispersed globule is carried out. The crosslinking of dispersed phase and dispersion medium is achieved by heat or employed with crosslinkers (i.e., formaldehyde, glutaraldehyde, di-acid chloride). Thermosensitive products are not suitable for this technique due to denaturation of product while subjected into heat.

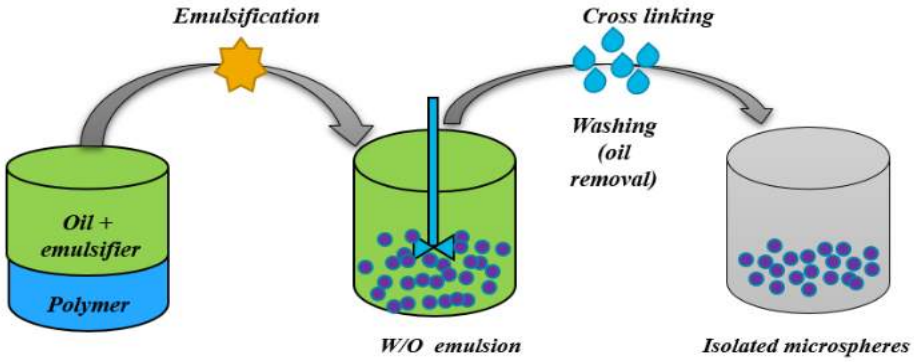


Figure 03: Single emulsion solvent evaporation

1.3.2 Multiple-Emulsion Technique (w/o/w)

Multiple-emulsion or double-emulsion technique is selected for the efficient incorporation of water-soluble peptides, proteins, and other macromolecules. This method allows the encapsulation of water-soluble drugs with an external aqueous phase when compared to nonaqueous methods as the w/o/w solvent evaporation or organic phase separation. On short, the polymers are dissolved in an organic solvent and emulsified into an aqueous drug solution to form a w/o emulsion. This primary emulsion is re-emulsified into an aqueous solution containing an emulsifier to yield multiple w/o/w dispersion. The organic phase plays as a barrier between the two aqueous compartments, preventing the diffusion of the active material towards the external aqueous phase. Microspheres fabricated by the (w/o/w method shown various morphological character like porous or nonporous external polymer shell layers enclosing hollow, macro-porous, micro- porous internal structures, depending on different parameters.

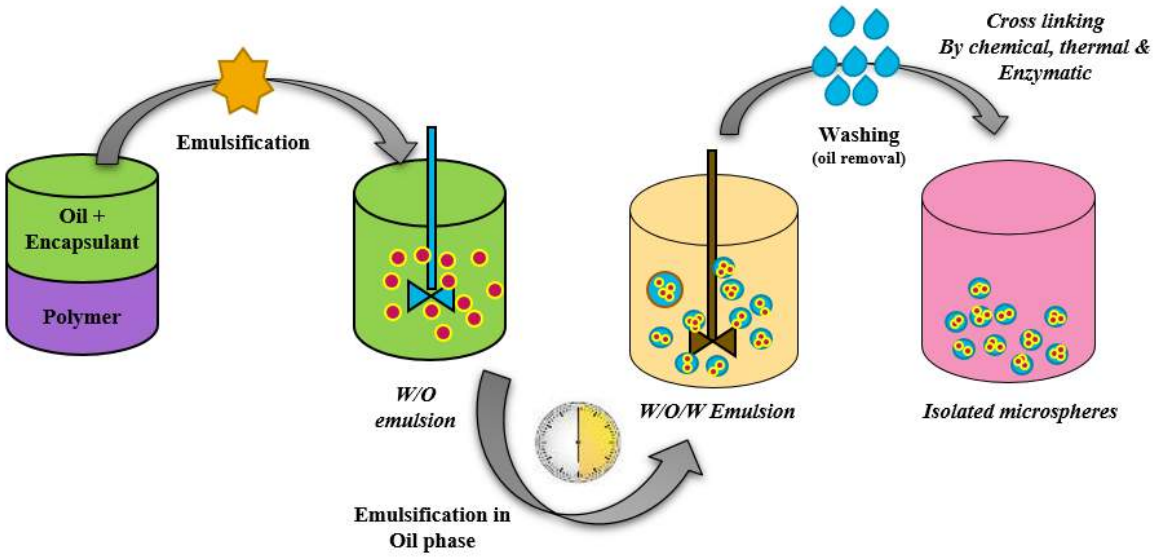


Figure 04: Double emulsion solvent evaporation

1.3.3 Coacervation/Phase Separation

Coacervation employs the separation of coating material of polymeric solution and wrapping of that phase as a uniform layer around suspended drug particles.

Principle: Drug materials are dispersed with the polymer solution and incompatible polymer is added to the system, which makes the separation of first polymer and engulf the drug particle. Under this method microsphere can be fabricated by using below steps

a. ***Formation of three immiscible chemical phase:*** In this phase the core material is dispersed in solution of coating polymer, the solvent for polymer being liquid manufacturing vehicle phase.
b. ***Deposition of coating polymer on core material*** &: In this phase the coating material subjected to deposit around drug core material. There absorption at interphase between core material & liquid vehicle phase will occur.
c. **Rigidization of coating material:** This phase will be done by applying thermal, cross linking or desolvation techniques to form microspheres.

Suitable pharmacological drug class: Anti-inflammatory, Analgesic, Antibiotics and anti-hypertensive can be employed.

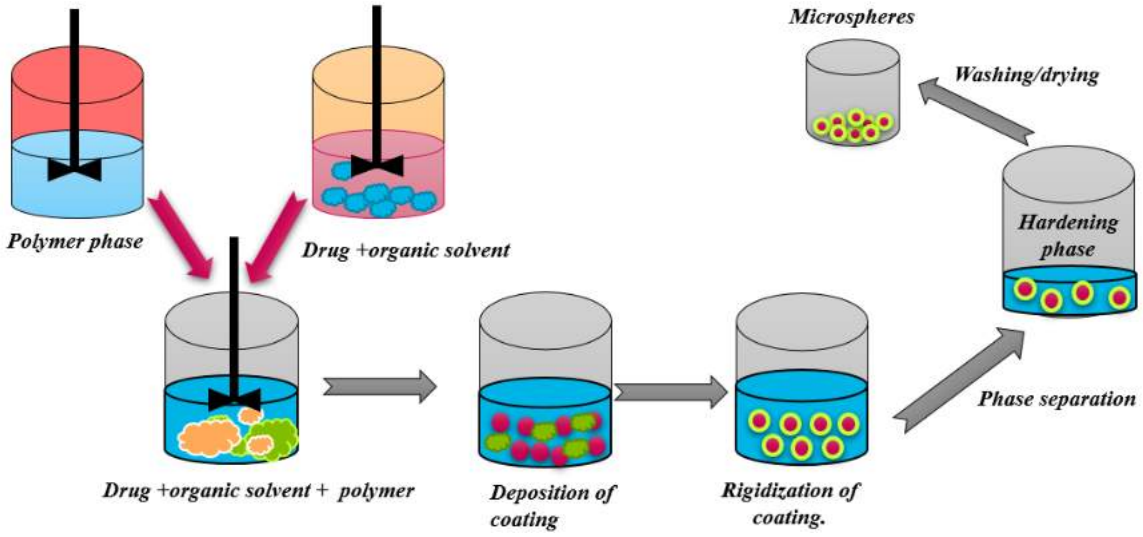


Figure 05: Coacervation technique

1.3.4 Polymerization Technique

Polymerization process defined as *reacting monomer molecules together under the influence of catalyst in a chemical reaction to form polymer chains.*

Mechanism: Monomer or mixture of monomer are subjected into heat with the catalyst to initiate polymerization. While applying heat the polymer will Mould the micro-spheres, drug loading is done during polymerization process.

It is offered by different methods such as suspension, precipitation, emulsion and micellar polymerization process. "Suspension polymerization" is carried out by applying heat to the monomer or monomer mixture as droplets dispersion in a continuous phase.

The droplets contain a catalyst as initiator. "Emulsion polymerization" allows the presence of catalyst in the aqueous phase, which later diffuses the surface of micelles.

1.3.5 Interfacial Polymerization Method

Interfacial polymerization technique is one in which two monomers, one oil-soluble and the other water-soluble, are employed and a polymer is formed on the droplet surface. The method involves the reaction of monomeric units situated at the interface existing between a core material substance and continuous phase in which the core material is dispersed.

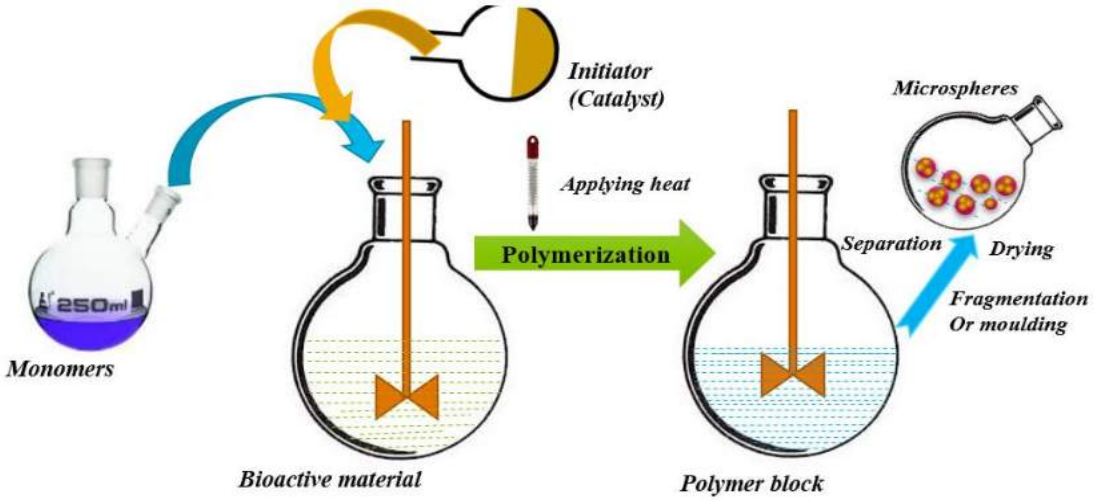


Figure 06: Polymerization technique

1.3.6 Spray Drying

In Spray Drying technique initially polymer is getting dissolved in a suitable volatile organic solvent such a dichloromethane, Acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of microspheres. Micro particles are separated from the hot air by means of the cyclone separator while the residual of solvent is eliminated by vacuum drying. Feasibility of operation under aseptic conditions can be offered. This process is rapid, and this leads to the formation of porous micro particles.

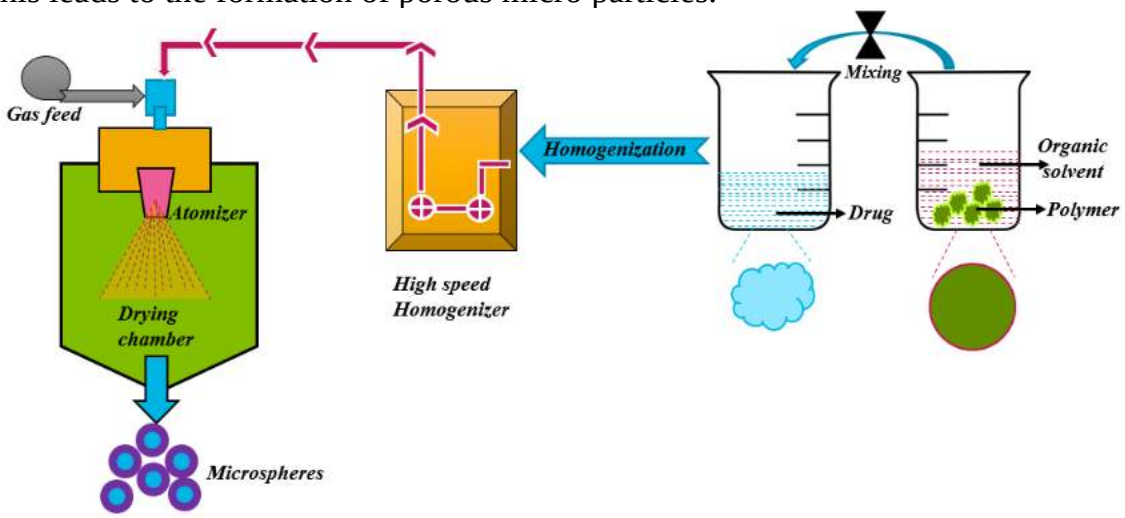


Figure 07: Spray drying technique

1.3.7 Solvent Extraction process

In this method preparation of microparticles, involving with removal of the organic phase by extraction of the organic solvent. Isopropanol can be use as water miscible organic solvents. By extraction with water, Organic phase is removed. Hardening time of microsphere can be reduce by this method. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer.

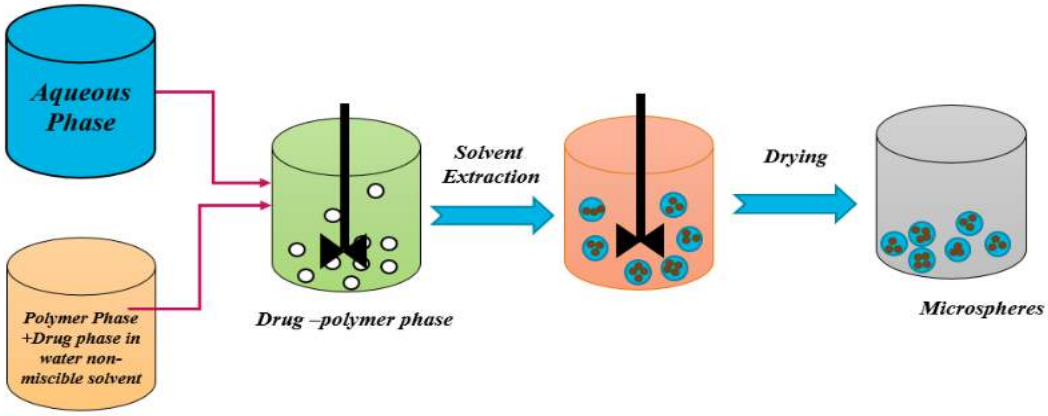


Figure 08: Solvent extraction process

1.4.0 RECENT ADVANCES IN MICROSPHERE TECHNOLOGY

1.4.1 Solvent Exchange method

The solvent exchange encapsulation technique principle about on interfacial mass transfer between an aqueous drug solution and a water-insoluble polymer organic solution upon contact to form reservoir-type microcapsules. The surface tension difference and the incompatibility between the drug aqueous phase and the polymer solution phase are the reaction driving forces. Aqueous micro-drops containing drugs and micro-drops containing polymers are produced rapidly using ink-jet nozzles controlled by a piezoelectric transducer. The two ink-jet nozzles are assisted to cause a mid-air collision between the two micro-drops. Solvent exchange carried upon contact of the two micro drops resulting in reservoir-type microcapsules which are collected in an aqueous bath. [(11)]

1.4.2 Microfluidic Flow-Focusing Method

Microspheres prepared using conventional emulsification techniques, such as sonication or homogenization, generally have a very broad size distribution, which results in:

a) potential batch-to-batch variations,

b) Different polymer degradation rates

c)Different drug release profiles.

Microfluidic flow focusing produces uniform-sized drug loaded droplets to obtain microspheres with narrow size distribution procedure used to fabricate monodisperse polymer microspheres via this method.

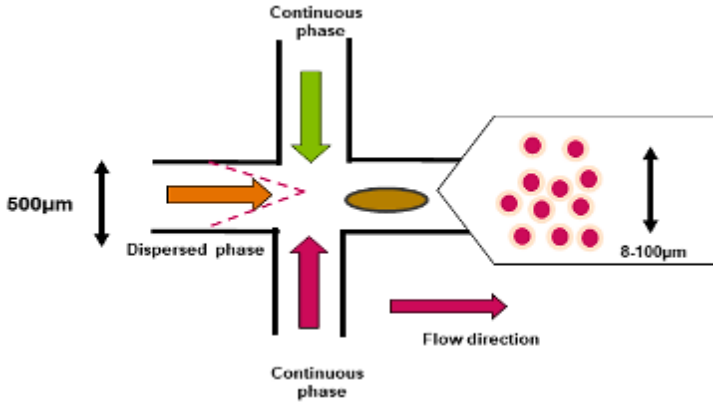


Figure 09: Microfluidic Flow-Focusing Method

1.4.3 Supercritical Assisted Atomization

Supercritical assisted atomization (SAA) is an alternative to the conventional jet-milling process. During the process, supercritical carbon dioxide is dissolved in a liquid drug loaded solution and this mixture is then sprayed through a nozzle. Microspheres will be formed as a result of atomization. Compared to the conventional jet milling or spay drying technology. This technique is highly preferable for thermolabile compounds because the operation temperature is very close to room temperature. In addition, SAA provides better control over the particle size. [(12)]

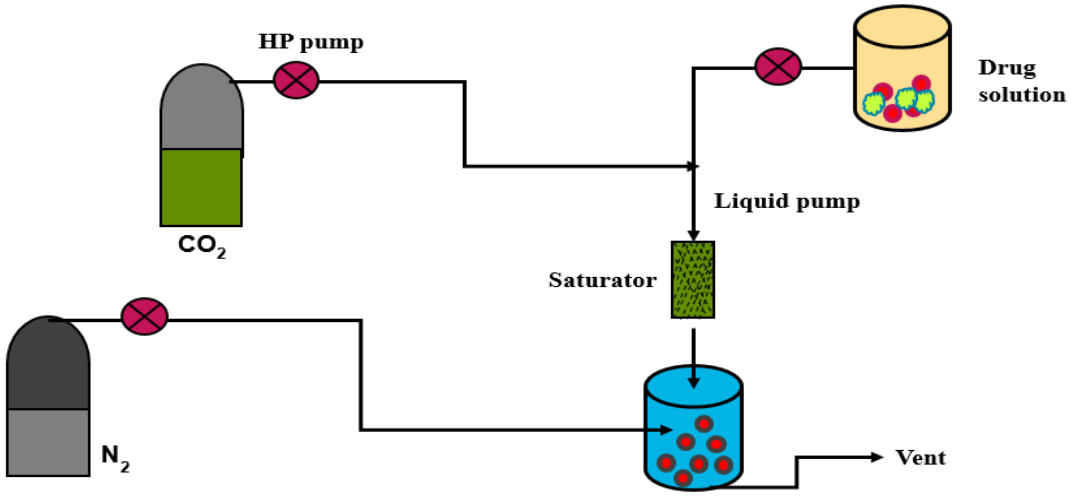


Figure 10: Supercritical assisted atomization technique

1.5.0 ROLE OF ADDITIVES IN PHARMACEUTICAL FORMULATION

Pharmaceutical additives are secondary constituents present in both pharmaceutical formulation and over the counter drug formulations. Additives are categorized on the bases of their function and interactions influencing drug administration due to their chemical and Physico-chemical properties. The major categories are the Ointment bases, Emulsifier, coating additives, Sweetener Flavorants, antioxidants, consistency or viscosity enhancers, and disintegrating materials. Few additives have serves more than one function. Additives carry out a key function in drug development operation in the formulation of stable dosage forms and in their administration. Pharmaceutical additives employed to take delivery of the dosage form with ease, to enhance the stability of active ingredients, to fill a dosage form (Filler), or to serve as preservatives for enhancing the shelf life of the product or Active Pharmaceutical Ingredient.

Pharmaceutical additive's functional roles in dosage form and on drug substance are: -

- To protect the physical and chemical entity of dosage form.
- To enhance the drug product storage and maintain the consistency until completion of shelf life period.

- To improve stability of finished product.
- To make better patient acceptance.
- To make more palatable.
- To enhance the bioavailability of drug product.
- To maximize the product efficacy and longer life cycle.
- To increase the product life cycle with expected or claimed time period.

1.5.1 Current regulatory status of new additives

According to the health authority guideline (USFDA) "Guidance for Industry: Non-clinical Studies for the Safety Evaluation of Pharmaceutical Excipients, May- 2005"addresses the aspect of approval process with the requirements for new additives or novel additives for the first time of pharmaceutical drug products or have a new route of administration. USFDA documented the inactive ingredient database (IID) has the approved products list and route of administration with acceptance level of concentration (dosage).

1.5.2 Additives In Controlled Release Solid Dosage Forms

Controlled release (CR) dosage is formed by using polymeric additives which coat around a drug core by microencapsulation or as a matrix in which the drug is embedded. It includes water-soluble resins (e.g. gelatin, starch, polyvinyl pyrrolidone, and water-soluble celluloses), water-insoluble resins (e.g., polymethacrylate, silicones, and water-insoluble celluloses), waxes and lipids (e.g., paraffin, beeswax, stearic acid), enteric resins (e.g., shellac cellulose acetate phthalate). Surfactant like tween 20 and PEG additives have been used in microencapsulation of macromolecules for various effects. [13]

1.6.0 POLYMER SCIENCE IN MICROSPHERES

A polymer is a large molecule made up of chains or rings of linked by repeated subunits of monomers. Polymers usually have high melting and boiling points. Because the molecules consist of many monomers, polymers tend to have high molecular masses (Long chain organic molecules assembled from many smaller molecules called as monomers).
In microsphere formulation incorporation of polymers are retard the release of drug by modifying the release rate or release pattern from the drug product. Hence the consolidation of polymers considered.

1.6.1 Classification of polymers

Type of polymers	Examples
Natural Polymers	Agarose, Chitosan, Carrageenan, Gelatin, Pectin, Tragacanth, Sodium alginate, Xanthum gum.
Synthetic polymers	HPMC, Sodium Carboxymethyl cellulose, Polyvinyl ethers, polyvinyl esters Polycarbonate, Poly vinyl alcohol, Polyamides, Poly alkylene glycols, Poly methacrylic acid, PMMA, Methyl cellulose, Ethyl cellulose, HPC, HPMC, Methyl cellulose.
Biodegradable polymers	Poly lactides [PLA], Poly(lactide-coglycolides) [PLGA], Poly caprolactones, Poly anhydrides, Polyethylene oxide, Poly alkyl cyanoacrylates, Poly orthoester, Poly(glycolides) [PGA], Poly phospho esters, Poly phosphagens.
Biocompatible polymers	Ethylene glycol, Polyvinyl acetate, Hyaluronic acid esters.

1.6.2 POLYMERS USED IN CRDDS

Controlled drug delivery systems have been developed markedly to overcome the troubles associated with conventional dosage form. The common merits of such delivery systems are that the administration dosage frequency can be reduced by controlling the complete dose of the drug with CR polymeric matrix in such a way that the matrix will release the drug for a longer period with pre-determined rate and led to better patient compliance. Improved stability, increased bioavailability, decreased toxic effect of the drug due to repetitive and chronic use of the drug. Sometimes, the total use of the drug may be minimized in a comparison to conventional dosage forms.[14]

1.6.3 Duties of Polymers in CRDDS

CR formulation of any drug can be fabricated by mixing it with an ideal concentration of polymer, which retards down the release of the drug in the medium by the below referred mechanisms:

- Dissolution-controlled system
- Diffusion-controlled system

Preferably, CR formulation should be designed in such a way that the optimum concentration of the drug required for the therapeutic effect should reach its C_{max} in systemic circulation and maintain the same concentration for a long period of time.

Polymers based on solubility	• **Hydrophobic polymers**
	  • Ethyl cellulose, Eudragit, HPMCP 55
	• **Hydrophilic polymers**
	  • Sodium CMC, HPMC, Gum.

Polymers based on molecular force	• **Elastomers**
	  • Polyacrylamide rubber, silicone etc
	• **Thermosetting**
	  • Epoxyresin, polyurethane, duroplast.

Polymers based on polymeriazation.	• **Condensation polymers**
	  • Nylon, Bakelite.
	• **Addition polymers**
	  • polyethane, polypropylene, PVC,Teflon .

1.6.4 Natural Polymers

Natural polymers have become the prime choice for the development of drug delivery systems due to their highly compatible and biodegradable nature as collated with synthetic polymers. These polymers can be acquired from various natural resources like animals and plants, and from marine and microbial origin. [15]

1.7.0 FACTORS INFLUENCING BIODEGRADATION OF POLYMERS

Biodegradation is referred to as the "process of modification in such a way that leads to the formation of a simple molecule that could easily be cleared from the body". Biodegradation in the living system may either be due to hydrolysis or by enzymatic action. There are several factors that may affect the process of degradation. They are described below: -

Molecular Weight

Higher molecular weight is essential for the mechanical strength of drug product and increased mechanical strength delays the biodegradation. But at the same time, few

polymeric materials like polycaprolactone are rapidly degraded in biological condition due to the presence of hydrolyzable groups. [16]

Chemical composition

The chemical composition of the polymeric system may have an impact on the biodegradation. In generally, if a molecule is water soluble then it will be easily hydrolyzed. But if impart of the hydrophobic character to this molecule the degradation via hydrolysis may be decreased. [17]

Distribution of Repeat Units in Multimers

Presence of another monomer unit and branching both can alter the biodegradable Property of polymers. Studies show that succinoyl substitution to the polymer helps in improving the biodegradability. At same time, branching and position of the double bond in the polymeric system may also alter the degradation property. [18]

Presence of Chain Defects

The biodegradable character of any polymer is markedly impact by chain length and any defect in chain-like presence or absence of chirality, which may also because of any unexpected group or unit present on the carbon. Incorporation of any hydrophilic group or absence of double or triple bond may increase the hydrophilicity of the molecule. Incorporation of hydrophilicity, hydrophobicity, or chirality may have a great impact on the biodegradable behavior. [19]

Presence of Ionic Groups

Polymeric degradation may also be altered by the pH of media, where biodegradation must occur by changing the polymeric chemistry by ionization. Higher degradation reported that water uptake by the polymer decreases initially in the presence of ionic solution, but, with the release of degradation products, degradation and erosion of polymer increase in the presence of ions. [20]

Morphology

Crystallization of the polymer own a regular structure with adjacent packing of molecules and increases the chances of intermolecular attractions, and this close packing is important for stability against biodegradation due to poor permeability of the solvent through it. Semi-crystalline and amorphous system doesn't have such close packing and swells easily in presence of a solvent, leading to comparatively easy degradation. [21]

Shape of the Polymer

Biodegradation of polymers may be increased by altering the shape, that increases the surface for interaction with microorganisms or enzymes. Effect of shape on biodegradation is not very significant in the case of easily bio-degrading plastics while the shape has a significant effect on biodegradation in the case of slowly biodegrading plastics. [22]

Physicochemical Factors

Physico-chemical factors like presence of ionic group in the polymeric molecule and pH of the environment also have a significant effect on the biodegradation process. Presence of ionic group or charge on the polymeric surface is an important factor for surface modification. Various bonds present in the bio-degradable polymers are either fragments at pH (ester bond breaking at more than pH 6.8) or by enzymes such as glucosidase, azo reductases, which are active at the specific pH. [23]

1.8.0 SURFACTANT SCIENCE IN CRDDS

The essential of surfactants in the formation of nano or micro particles is due to its high effect on the dispersion. microemulsions, as non-equilibrium systems, present characteristics and properties which depend not only on composition but also on the preparation method. Surfactants functions a major role in the formation nanotechnology formulations by lowering the interfacial tension, prevention of coalescence for newly formed drops. [24]

HLB is a dimensionless parameter for surfactants which is known as a time saving guide to surfactant selection. Also, the HLB value of a surfactant plays an important role in controlling drug entrapment efficiency.

HLB range is from 0 to 20 for nonionic surfactants; a low HLB (b9) refers to a lipophilic surfactant (oil soluble) and a high HLB to a hydrophilic (water soluble) surfactant. Surfactants with an HLB number between 3 and 8 are compatible with preparation bilayer surfaces and refer to water-in-oil (W/O) emulsifier. Also, oil-in-water (O/W) emulsifiers exhibit HLB values within the range of 8–18.

Non-ionic surfactants are preferably one of the best polymeric nanocarriers with a wide role in controlled, sustained, targeted and continuous drug delivery. generally, surfactants are classified according to their polar head group.

A non-ionic surfactant has no charge groups in its head. The head of an ionic surfactant has a net charge and is called an anionic surfactant. Examples of such surfactants include

fatty acid salts (“soaps”), sulfates, ether sulfates and phosphate esters. If the head charge is positive, it is called a cationic surfactant.

If a surfactant contains a head with two oppositely charged groups, it is termed as a zwitterionic (amphoteric) surfactant. Cationic surfactants are also frequently irritant and sometimes even toxic; therefore, their application in drug delivery is more limited than the three other classes of surfactants.

Non-ionic surfactants are a category of surfactants which have no charge groups in their hydrophilic heads. Therefore, in solutions, nonionic surfactants can form structures in which hydrophilic heads are opposite to aqueous solutions and hydrophilic tails are opposite to organic solutions.

Surfactant class	Examples
Non-ionic	Polyoxyethylene alcohol
	Polyoxyethylene glycol alkyl ethers (Brij)
	Alkyl ethoxylate
	Alkyl phenol ethoxylate
	Fatty acid alkanolamides
	Propylene oxide-modified polymethyl siloxane
	(EO = ethylene oxy, PO = propylene oxy)
Anionic	Stearate
	Soap
	Alkyl benzene sulfonate
	Alkyl sulfates
	Ether sulfates
	Alkyl ether sulfate
Cationic	Lauryl amine
	Trimethyl dodecyl ammonium
	Cetyl trimethylammonium
	Alkyl diamine salt
	Benzyl alkyl dimethyl ammonium salts
	Alkyl quaternary ammonium salts
Zwitterionic	Dodecyl betaine
	Lauramido propyl betaine
	Cocoamido-2-hydroxypropyl sulfo betaine
	Alkyl imidazoline
	Alkyl betaines
	Sulfur-containing amphoteric

1.9.0 PHYSICOCHEMICAL CONSIDERATION IN GIT ABSORPTION OF DRUGS

Drug absorption is the amount of drug that enters the systemic circulation as unchanged form through various routes of drug administration. According to pharmaceutical term drug absorption can be defined as the "Process of movement of drug from the site of administration to systemic circulation." [25]

Sequence →	**Absorption**			**Metabolism**	**Distribution**	**Excretion**	
Region →	**Stomach**	**Intestine**		**Liver**	**Blood**	**Kidney**	
Barriers associated to specific regions →	***Stability:*** Acidic	***Stability:*** Acidic, Enzymatic condition ***Solubility:*** Aqueous, GI fluid solubility	***Permeability:*** Passive & Efflux	Phase-I, Phase-II reaction Biliary excretion Uptake Efflux CYP450 interaction	Protein binding & Enzymatic stability	Renal extraction Excretion Secretion ***Distribution*****:** Passive, Efflux, BBB permeability	**BBB**

1.10.0 MECHANISM OF GIT ABSORPTION OF DRUGS

Drugs are absorbed through the gastrointestinal tract when its administered orally. It works based on the mechanism of following categories.

- Passive transport
- Active transport
- Specialized transport

Passive transport

It is the movement of drugs across the cell membranes without the requirement of any form of energy. Passive transport can be of two types, namely passive diffusion and facilitated or carrier-mediated diffusion. Passive diffusion is the primary mechanism through which wide of the drugs are absorbed. Diffusion is described by Fick's law, which says that the rate of diffusion is proportional to the concentration gradient.

$$R = DA\,\Delta C/\Delta X$$

Where,
R: Rate of diffusion in moles
A: Membrane area

ΔC: Concentration gradient between the two sides of membrane
ΔX: Membrane thickness
D: Diffusion coefficient.

Facilitated or carrier-mediated diffusion

Facilitated or carrier-mediated diffusion takes place with the help of membrane proteins. These membrane proteins are known as "permeases". A typical example of a compound that is transported by this type of diffusion is glucose. Like passive diffusion this type of transport doesn't need any energy. There is a change when compared to passive diffusion that this process can be saturated as the permeases can be used fully at concentration, and after that enhancing the concentration will not help in increase in the diffusion rate, which is the rate-limiting step in the process of absorption.

Active transport

Active transport required energy to make it absorption. Active transport is possible from lower concentration to higher concentration, unlike diffusion mechanism. Adenosine triphosphate (ATP) hydrolysis provides the energy required for this process. Active transport is selective in a sense that drugs structural similarities with endogenous substances that are transported through this process are benefited. These drugs are usually absorbed from specific sites in the small intestine. Active transport is broadly classed into two types, namely primary and secondary. Primary or direct active transport uses metabolic energy directly, while secondary active transport, also known as coupled transport or cotransport uses electrothermal potential created by the ions across the membrane.

Specialized transport

Macromolecules are sometimes not able to cross the membranes either by diffusion or active transport as the pores in the membrane are too small for them to cross. In these cases, the molecules are taken up by the process known as cytosis. In this process membrane forms envelop surrounding the larger molecule or particles. There are three main variants of this process that occur in the cells. They are

- ***Phagocytosis:*** It occurs when the cell engulfs and internalizes a solid particle or cell.
- ***Pinocytosis:*** It occurs when a large volume of extracellular fluid is taken as vesicles into the cells.

- ***Receptor-mediated endocytosis:*** It happens with the help of receptors on the cell surface to which the drug adheres and is then taken up into the cell.

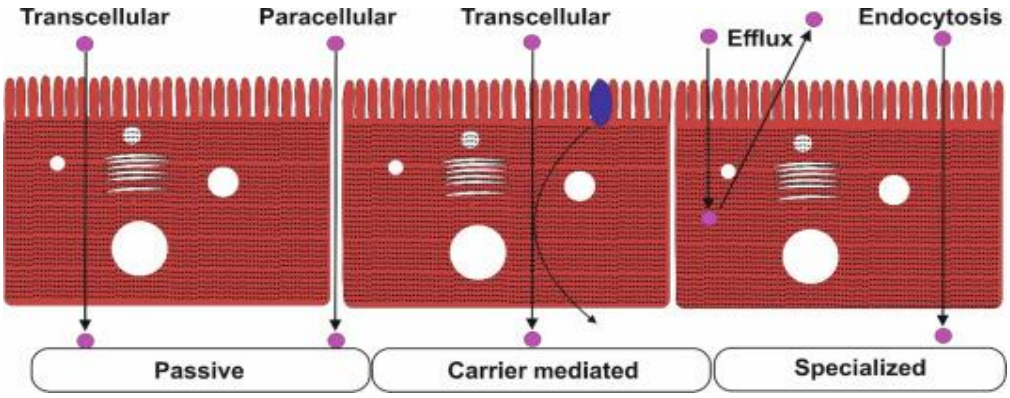


Figure 11: Mechanism of drug absorption schematic representation

1.11.0 DRUG RELEASE MECHANISM

Drug-release behavior is an important factor for polymer Novel drug delivery aspect, which is directly related to drug stability and therapeutic results, as well as formulation development. General term of release mechanism is in referring to the process that determines the rate of release, i.e. swelling, drug dissolution, erosion and polymer–drug interactions. Thus, diffusion and biodegradation are the process of drug release. In more cases, rapid drug release from polymer nanoparticles, called " burst release", can be observed initially. [(26)]

The drug may be released by diffusion through water-filled pores, and the rate of pore formation may be the rate controlling process. Polymer erosion, which is determined by the rate of hydrolysis, probably determines the rate of pore formation, although the absorption of water also results in pores. The processes defining the way in which the drug is released will be called the true release mechanisms, and the processes that control the release rate will be called rate-controlling release mechanisms.

S.R.No	Mechanism of Drug release
1	Diffusion through water-filled pores
2	Diffusion through the polymer matrix
3	Hydrolysis
4	Erosion
5	Osmotic pumping
6	Water absorption/Swelling
7	Polymer–drug interactions
8	Polymer relaxation
9	Pore closure
10	Heterogeneous degradation
11	Formation of cracks or deformation
12	Collapse of the polymer structure

When the drug is delivered using an microparticle delivery system, effectiveness is affected by parameters such as the particle size, release process from the particle matrix. The smaller the particles, the larger the surface area-to-volume ratio; therefore, most of the drug associated with small particles would be at or near the particle surface which leads to faster drug release. In contrast, larger particles have large cores, which allow more drugs to be encapsulated per particle and give slower release. Thus, control of particle size provides or regulates the drug release rates.

Polymer factors	Encapsulated substances	*in-vitro* condition	Sphere product
Drug: Polymer ratio	Nature of drug	Temperature	Shape
Molecular weight	Nature of polymer	Stirring speed	Size
Nature of drug	Drug load efficiency	Release medium composition	Porosity
-	Characteristics of additives	pH	Density
-	Surfactant concentration	Osmolality	-

The polymer coating acts as a drug release barrier /Release retarder hence, the drug solubility and diffusion in or across the polymer membrane becomes a determining factor in drug release. The release rate can also be affected by ionic interactions between the drug and secondary ingredients. If polymer-encapsulated drug interacts with auxiliary ingredients, a less water-soluble complex may form causing a slower drug release that almost has no burst release effect

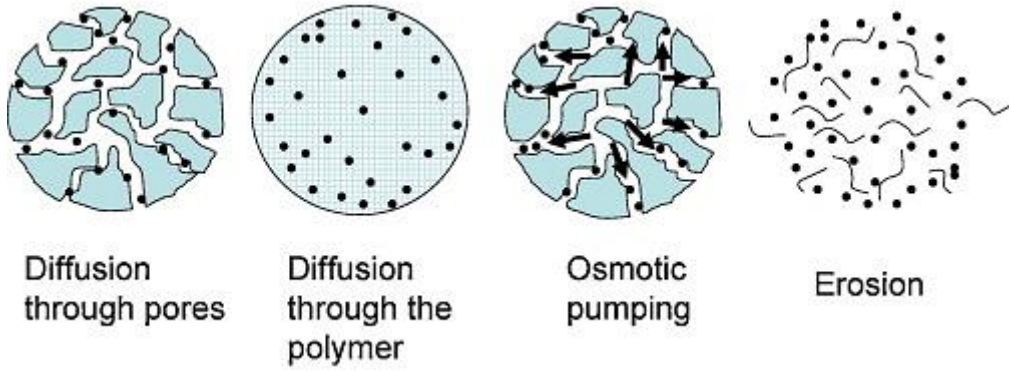


Figure 12: Mechanism of drug release from formulation

REVIEW OF LITERATURE

2.1.0 LITERATURES RELATED TO FORMULATION

S.Magdassi *et al.*, (2009) evaluated a new method to prepare nanoparticles of a poorly water-soluble drug of simvastatin by evaporation of all solvents from spontaneously formed oil-in-water microemulsions. In this method microemulsions containing a volatile solvent as an oil phase are converted into nanoparticles in the form of dry non-oily flakes by freeze-drying. It was found that after freeze-drying more than 95.0% of the drug was present in amorphous particles, smaller than 100nm.Tablets containing the flakes of simvastatin nanoparticles shown tremendous enhancement in dissolution profile compared with conventional tablets. [27]

M.Gambhire *et al.*, (2011) studied the solid lipid nanoparticle of Simvastatin to improve the oral bioavailability. Simvastatin SLNs were developed using compritol 888 ATO by pre-emulsion followed by ultrasonication process. Bioavailability studies were conducted in albino rats after oral administration of Simvastatin suspension and SLN. Stable Simvastatin SLNs having a mean particle size of 245 nm and % entrapment of 72.52% were developed. The relative bioavailability of Simvastatin and Simvastatin hydroxy acid from SLN were increased by ~164% and ~207% respectively, compared with the reference Simvastatin suspension. [28]

B. Agaiah Goud *et al., (2011)* developed mucoadhesive buccal tablets of Simvastatin using mucoadhesive polymers. The tablets were prepared by direct compression technique using carbopol-934, sodium carboxy methyl cellulose (Na CMC) and hydroxyl propyl methyl cellulose (HPMC) as mucoadhesive polymers. Formulations were evaluated for mass variation, hardness, friability, drug content, swelling studies, erosion studies, *in-vivo* residence time, *in-vitro* release studies in pH 7.0 phosphate buffer with 0.5% SDS.Formulation reported bio adhesive buccal tablets for Simvastatin with desired *in-vivo* residence time and controlled release about 08 hrs .[29]

Bathool *et al.*, (2012) developed the sustained release nanoparticles of Atorvastatin calcium solvent evaporation method using Chitosan as a polymer-determined amount of

drug and polymer were dissolved in suitable organic solvent DMSO and 2% acetic acid as an organic phase. This solution is added drop wise to aqueous solution of Lutrol F68 and homogenized at 25000rpm followed by magnetic stirring for 4hrs. Particle size of prepared nanoparticles was found to be in the range between 142 nm to 221 nm. *In-vitro* release study showed that the drug release was sustained up to 7 days. [30]

G.Abdelbary *et al.*, (2012) developed simvastatin containing self-Nano emulsifying systems (SNEs) to improve oral bioavailability of poorly water-soluble drugs. The *in-vitro* release results revealed that the developed SNE based tablets improved the release of simvastatin significantly, compared to commercially available tablets 1.5-fold increase in bioavailability.[31]

Bal *et al.*,(2012) developed Simvastatin/ Hydroxy propyl beta cyclodextrin (HPBCD) binary systems by co-grinding technique and formulated the binary system in oral mucoadhesive microcapsules by incorporation of hydrophilic sodium alginate and another plant seed mucilage dillenia (obtained from *Dillenia indica*) by using orifice gelation technique. Drug release from the formulation reported as 72.682% upto 12 hours in phosphate buffer of pH 6.8.Particle size about the range of 371.5 to 457 μm, and encapsulation efficiency of formulation exhibited 63.068 ± 0.002 to 99.083 ± 0.017%. [32]

Basuvan babu *et al., (2012)* developed single unit of oral sustained release dosage form Simvastatin have been prepared by the wet granulation method. The hydrophilic matrix was prepared with xanthan gum with additives MCC PH101. The extent of absorption of drug from the sustained release tablets was significantly higher than that for the marketed Simvastatin tablet because of lower elimination and longer half-life. Various pharmacokinetic parameters including AUC0-t, AUC0-∞, Cmax, Tmax, T1/2, and K_e were determined from plasma concentration of both Sustained and Immediate release tablets. [33]

S.D. Nath *et al.*, (2013) investigated Simvastatin-PLGA [poly (D,L-lactic -co-glycolide) microsphere formulation for extensive drug delivery. In this method PLGA microspheres are prepared by electro spraying method. Dichloromethane utilized as solvent for PLGA dissolution. The *in-vitro* experiments on drug loading and drug release behavior of the microspheres suggested a drug encapsulation efficacy >90%. The drug release reported from microspheres for more than 03 weeks. [34]

Athul P.V *et al.*,(2013) formulated and characterized nanosuspension of Simvastatin by high pressure homogenization method to improve its dissolution characteristics and therapeutic activity. The in-vivo pharmacological studies showed that the nanosuspension of drug has increased anti hyperlipidemic activity compared to the pure drug. (35)

D.P.Kulhari *et al.*, (2013) investigated to evaluate the in vivo potential of poly (amido) amine dendrimers (PAMAM) based simvastatin formulations as nanoscale drug delivery units for controlled release action of simvastatin. Drug-dendrimer complexes were prepared and subjected to FTIR.The cholesterol level was increased to 20.92% with pure simvastatin 11.66% with amine dendrimer, 11.49% with PEGylated dendrimer and 10.86% with hydroxyl dendrimer formulations. Dendrimer- Simvastatin formulation shown better pharmacokinetic performances than pure simvastatin suspension. (36)

A.S.Tulbah *et al.*,(2014) studied on the development of a dry powder inhaler (DPI) formulation of simvastatin and the effects of simvastatin on the respiratory epithelium. Micronized simvastatin prepared by dry jet-milling. Single dose of simvastatin Dry Powder Inhaler decreased mucus production after 4 days of dosing. This formulation was stable up to 9 months at 25°C/60% RH.(37)

Pabari *et al.*,(2014) studied spray dried solid dispersion (SDP) of crystalline form of simvastatin in a fast disintegrating matrix of super disintegrants to enhance rheological behavior, dispersibility, compatibility and compressibility of simvastatin oro-dispersible tablets. (38)

Tulbah *et al.*, (2014) investigated a pressurized metered-dose inhaler (pMDI) solution formulation of simvastatin. Simvastatin inhaler formulation prepared with ethanol (as a co-solvent). A pMDI solution formulation containing SV and 6%w/w ethanol was prepared and subjected for characterization study. The aerosol produced fine particle fraction of 30.77±2.44% and a particle size distribution suitable for inhalation drug delivery. Short-term chemical stability proven formulation to be stable at 4°C for up to 6 months, and 25°C the formulation was stable up to 3 months. (39)

R. Qi *et al.*, (2015) compared formulation effects of a dendrimer and a liposome formulation on the water solubility, transepithelial transport, and oral bioavailability of simvastatin .The study resulted in simvastatin oral absorption is better in simvastatin liposomes and markedly improved the C_{max} and oral bioavailability. Simvastatin liposomes provided much improvement an *in-vivo* oral absorption of simvastatin than the SMV/G5-NH2 Amine-terminated G5 PAMAM dendrimer complexes. (40)

Franceschinis *et al.* (2015) studied the influence of process variables on the properties of self-emulsifying granules, which are produced by using a high shear mixer. Simvastatin-loaded microemulsion was used as a binder and was dripped on powder mixture composed of 70% (w/w) MCC, 27.0% (w/w) lactose and 3.0% (w/w) PVP. Granules showed larger disintegration time due to oily bridges that decrease wettability of granules. The longer massing time results in higher disintegration time because consolidation of granules was more extensive. (41)

Tulbah *et al.*,(2015) evaluated the biological effects of Calu-3 epithelial cells in response to the delivery of simvastatin via solution pressurized metered dose inhaler (pMDI). Simvastatin shown the ability to penetrate the respiratory epithelium and convert into its active simvastatin hydroxy acid (SVA) metabolite. Simvastatin delivered by pMDI reduced production of IL-6, 8 and TNF-α from Calu-3 followed by stimulation with lipopolysaccharide (LPS). (42)

T.Terukina *et al.*,(2016) investigated two simvastatin loaded poly(lactic-co-glycolic acid) formulations of PLGA microspheres and PLGA nanospheres for bone regeneration treatment.Sustained release of PLGA microspheres of simvastatin exhibits the drug release about 30 days, although simvastatin distinctly released from PLGA nanospheres for 07 days. The variation of drug release pattern between two PLGA particles was confirmed by Korsmeyer-Peppas mathematical model. (43)

P. Gentile *et al.*,(2016) investigated about localized slow release of simvastatin from porous freeze-dried chitosan gelatin (CH–G) scaffolds by incorporated with simvastatin loaded poly-(DL-lactide-co-glycolide) acid microparticles (MSIMs). MSIMs at prepared using a single emulsion solvent evaporation method. Based on study outcome, controlled release of simvastatin capable to influence the hFOB cell proliferation and the osteoblastic differentiation after 11 days over time. (44)

K. Wang *et al.*,(2017) prepared Hollow carbonated hydroxyapatite microspheres of simvastatin sustained-release vehicles were manufactured through a novel and simple one-step biomimetic strategy. Firstly, hollow $CaCO_3$ micro-spheres precipitated through the reaction of $CaCl_2$ with $Na2CO_3$ in presence of aspartic acid and sodium dodecyl sulfate. Prepared hollow $CaCO_3$ microspheres modified into HCHAp microspheres with a controlled anion-exchange method. The HCHAp microspheres size range from 3.0 to 5.0

μm with a shell thickness of 0.5 to 1.0 μm. The HCHAp microspheres were loaded with simvastatin and it shown extreme drug-loading capacity and sustained release properties. (45)

D. Orgul *et al.*, (2017) developed a formulation of simvastatin loaded Nano structured lipid carriers for the treatment of diabetic wounds. Nanostructured lipid carriers are formulated via high shear homogenization process which are prepared by using blends of solid and liquid lipids. NLCs have nanostructures, improved drug incorporation/release properties and drug targeting capability. Formulation resulting NLC suspension contained spherical nanosized (110-158 nm) homogeneous particles (PDI< 0.16) with > 99.0 % encapsulation efficiency. (46)

Alina Porfire *et al.*, (2017) developed a lyophilized formulation of simvastatin loaded long circulating liposomes prepared by film hydration method. In the method design space confirmed with cholesterol concentration about 13.7 mm,4.13 % PEG, 0.92 and two homogenizations through 100 nm polycarbonate membranes utilized then freezing at -80°C before lyophilization was prepared in triplicate design.(47)

Syed Zaki Husain Rizvi *et al.*, (2019) developed solid lipid nanoparticles-loaded with simvastatin and vivo anti-hyperlipidemic activity in poloxamer-induced hyperlipidemia model. Nano-template engineering technique was followed to fabricate Simvastatin-Solid Lipid Nanoparticles'-SLNs demonstrated a sustained release from the lipid core of nanoparticles. SIM-SLNs significantly reduced the elevated serum lipids as indicated by ∼3.9 and ∼1.5-times decreased total cholesterol compared to the untreated control and Simvastatin dispersion treated hyperlipidemic rats. (48)

M. Yasaei *et al.*,(2019) developed a layered double hydroxide (LDH) based drug delivery system of simvastatin loaded with a series of ZnAl-NO3(CO3) LDHs using two different synthesis process such as direct coprecipitation and the ion exchange of nitrate. From an ion-exchange process, the loaded drug quantity into LDH was 7.9 and 2 wt. % for coprecipitation process obtained 16.2 &5.5 respectively. The drug release rates of 84% and 93% were reported in CO3-based LDH compared to that of 35% and 45% for NO3-based counterpart after 58 hrs. The NO3-based LDH system showed sustained drug delivery compared to that of CO3-based LDH with relatively lower drug loading. (49)

2.2.0 LITERATURE RELATED TO DRUG RELEASE

T. Tanigo *et al.*,(2010) demonstrated the sustained release delivery of bio-degradable hydrogel of gelatin with water-insoluble simvastatin. Simvastatin initially water-solubilized by gelatin grafted with L-lactic acid oligomer and mixed with gelatin, then chemical crosslinked to obtain gelatin hydrogels incorporated simvastatin. The hydrogel augments the simvastatin induced bone regeneration . (50)

P.A.Sonar *et al.*,(2013) demonstrated study about preparation of solid dispersion formulation of simvastatin by lyophilization utilizing skimmed milk as a carrier. To enhance the solubility of simvastatin, the optimum drug: carrier ratio of 1:9 suggested to enhance solubility nearly 30-fold as compared to pure drug. *In-vitro* drug release study exhibited a cumulative release of 86.69% as compared with 25.19% for the pure drug. Solid dispersion of Simvastatin using skimmed milk as carrier approach better for oral delivery of Simvastatin. (51)

J. K. Patel *et al.*, (2014) investigated about micronization of simvastatin by using supercritical anti-solvent technique. Simvastatin dissolved in Acetone, DMSO and ethanol with supercritical Carbon dioxide. Study reported the dissolution rate was increased after micronized simvastatin compared with pure simvastatin in distilled water, pH 1.2 buffer and pH 7.0 buffer. In vivo performance of the optimized formulation also evaluated in rats using pharmacodynamic marker parameters like serum total cholesterol (CH) and triglycerides (TG) for 21 days. Pharmacodynamic studies of micronized simvastatin revealed improved reduction in CH and TG values as compared with pure simvastatin indicating improved bioavailability. (52)

F.T.Karim *et al.*, (2014) carried out work of development of Self Emulsifying Drug Delivery System (SEDDS) of simvastatin. Oils and surfactants were screened out depending upon their solubilizing capacity. Among all the solvents, Capryol 90 and tween shown good solubilizing capacity. 7:3 (m/m) mixture of Capryol 90 and Tween-80 exhibited smallest microemulsion with particles size of 0.074 mm and drug release was 102% within 20 min. Ex vivo study of the SEDDS formulation was evaluated using guinea pig intestinal sac. Drug diffused from formulation markedly higher than pure drug (p50.001). (53)

R.Laitinen *et al.*, (2015) studied dissolution property of co-amorphous drug :amino acid mixtures, which was spray-dried form aqueous solutions by using a surface-active agent sodium lauryl sulfate (SLS) as a solubilizer for the poorly water-soluble drug simvastatin. Simvastatin and lysine (LYS) were dissolved at a 1:1 molar ratio in 0.5% or 5.0 % SLS water solutions, which were then spray dried to obtain the formulation. Simvastatin from 5.0 % SLS formulation shown extreme dissolution property in pH 7.2. (54)

T.Terukina *et al.*,(2016) developed a formulation of simvastatin loaded PLGA microspheres /carbonated hydroxyapatite (CHAP) composite and investigated the effect of simvastatin release from that composite in comparison with a SIM/CHAP composite used as a control. The SPLGAMs and SPLGAMs/ CHAP composites produced the sustained release of Simvastatin for 1 month and Simvastatin/ CHAP composite released Simvastatin for 2 weeks. From the results, it recommends that the SPLGAMs/CHAP composite could release simvastatin controllably and increase proliferation and differentiation of pre-osteoblast murine cell line cells more effectively than the Simvastatin/CHAP composite. (55)

F.Ungaro *et al.*, (2017) demonstrated formulation of microparticles of poly(lactic-co-glycolic) acid incorporating simvastatin either as lactone or as hydroxy acid form by using spray-drying technique. While SVA-loaded microparticles released the drug in three days and long-term release of SVA could be obtained from SVL-loaded microparticles. In SVL was promptly transformed to the osteogenic active SVA during release. Invitro release are evaluated with 3 mg of dried microparticles were suspended in 2 mL of phosphate buffer saline (PBS) at pH 7.4 (120 mM NaCl, 2.7 mM KCl, 10 mM phosphate salts) containing 0.05% (w/v) of sodium azide as preserving agent. (56)

F. Qiao *et al.*, (2017) developed simvastatin loaded PLGA dimpled microspheres by single emulsion solvent evaporation method. The Simvastatin loaded PLGA dimpled micro-spheres were dispersed in silk fibroin solution (1 mg/mL, pH = 9.16).Then ethanol was injected dropwise into suspension and stirred at 800 rpm 1 hr. under ambient condition. The particles were centrifuged and cross-linked by glutaraldehyde (2%,w/v) for 1 h followed by washed thrice. Final product subjected into lyophilizer. The SIM-PLGA loaded dimpled microsphere exhibits 24.5%(drug loading: 17.0%) and 18.7% (drug loading,6.7%), respectively. (57)

S.Yasasvini *et al.*, (2017) developed prolonged release drug delivery system by loading Simvastatin-chitosan microparticles into polyvinyl alcohol (PVA) hydrogels by ionic gelation method to enhance wound healing efficiency. Hydrogels containing 2.5 mg equivalent dose of Simvastatin microsphere shown extreme cumulative percentage drug release of 92% at the end of 7th day. The *in-vitro* drug release of 5% PVA hydrogels loaded with 2.5mg, 5.0mg and 10 mg dose of Simvastatin reported as steady state and constant release of drug to reach the extreme of 92% (754 µg) at the end of 7 days. About 60%(650 µg) and 36%(575 µg) of the drug was released from the medium and high dose hydrogels distinctly. (58)

S. Taymouri *et al.*, (2017) developed hydroxypropyl methyl cellulose and chitosan gel containing polymeric micelles loaded with simvastatin and evaluated its wound healing properties in rats. Formulation prepared by film hydration method. Particle size and release efficiency more affected by hydration temperature. The optimized formulation suggested by desirability of 93.5% was prepared using 1.0 mg of Simvastatin and 10.0 mg of copolymer, dichloromethane used as organic solvent, hydration time of 45 min and hydration temperature of 25°C. The release of the drug from Nano micelles shown rapid release in the first stage followed by a sustained release for 96 hrs. (59)

Yamaki *et al.*, (2018) synthesized Simvastatin-arginine complex in the solid state by freeze drying method. The aqueous solution of arginine saturated with simvastatin followed by incubated in water bath at 37°C for 72 hours. After equilibrium, the suspension filtered via 0.5 µm membrane filter. The filtrate further subjected into lyophilization for solid complex formation. Simvastatin Arginine complex were formed at the proportion of 1:2. The mean particle diameter ranged from 5.9 ± 1.34 to 14.2 ± 2.65 µm. Release rate of simvastatin reported only 17.3% throughout the dissolution period of 800 min. (60)

Antonella Barone *et al.*, (2019) assessed this potential of simvastatin to support the therapy against melanoma by development of topical adhesive film which is composed by chitosan coated Nano structured lipid carriers. Optimized Ch-NLC exhibited particle size of 108 ± 1 nm, a polydispersity index (Pdi) of 0.226, a zeta potential of 17.0 ± 0.6 mV, and entrapment efficiency of 99.86 ± 0.08%, with the loading of 14.99 ± 0.01%. The assessment stated, concentration of Simvastatin found in the receptor medium of Franz cells at 48 h largely exceeded. Topical Simvastatin-Ch-NLC films are capable to provide an in situ extended drug delivery for treatment of melanoma skin lesions.(61)

X. Li *et al.*, (2019) developed an injectable microsphere hydrogel system of simvastatin was loaded PLGA microspheres were prepared by using an emulsion process, and the drug loaded PLGA microspheres were further entrapped in a gelatin hydrogel to form an injectable microsphere hydrogel system. A rat tooth extraction socket model was generated, and the simvastatin-loaded microsphere-hydrogel composite was injected in the defect area. At 1, 2, 5, and 8 weeks after the surgery. Simvastatin-loaded microspheres and hydrogel containing simvastatin-loaded microspheres released simvastatin for 4 weeks and shown loading capacity of 10.2% and an encapsulation efficiency of 39.0% & PSD about 8.0 µm. [62]

2.3.0 LITERATURE RELATED TO POLYMERS IN FORMULATION

C.Lorenzo *et al.*, (2003) studied osteogenic and osteo-inductive effect with the combination of simvastatin, poloxamine Tetronic 908 (T908) and a cyclodextrins (aCDs) in a supramolecular network.Incorporation of 5% aCDs transforms dilute T908 solutions (as low as 2% copolymer) into gels, enhances the osteo-inductive activity of T908, and provides simvastatin sustained release for more than one week period of time.[63]

Ambike *et al., (2005) f*ormulated the surface solid dispersions of Simvastatin to enhance the aqueous solubility and dissolution rate to facilitate faster onset of action. Surface solid dispersions of Simvastatin with two different superdisintegrants in three different drug–carrier ratios were fabricated by a co-evaporation method. Surface solid dispersions were characterized by DSC, PXRD ,SEM and infrared spectroscopy (IR) and evaluated for drug content, saturation solubility, pH-dependent solubility, solubility in biorelevant media *in-vitro* dissolution, and *in vivo* studies by a Triton-induced hypercholesteremia model in rats., which resulted in an increased dissolution rate of Simvastatin. [64]

J.H. Jeon *et al.*,(2007) reported the association polymer system of cellulose acetate phthalate and Pluronic F-127 used in simvastatin daily injection for mimicking the intermittent drug release. Simvastatin CAP/PF-127 microspheres fabricated by water–acetone–oil–water (W/A/O/W) triple emulsion process.60 mg dosage forms serum concentrations of simvastatin and simvastatin acid are 18.7±4.7 and 3.5±0.5 ng/mL, respectively. Furthermore, the half-life of simvastatin acid about 5.9±0.3 h . The release of simvastatin acid determined at a rate of approximately 36.5 ng/h. [65]

R. Boppana *et al.*, (2009) developed a novel interpenetrating network hydrogel beads of simvastatin with sodium carboxymethylcellulose (4% w/v) and egg albumin, via ionotropic gelation and covalent cross-linking method. Prepared beads treated with higher concentration of glutaraldehyde ,hence the drug release more slowly. The ionically cross-linked beads shown potential of drug release upto 7 hr., whereas the drug release was extended up to 12 hr. by utilization of dual cross-linked beads. [(66)]

M.Vidyavathi *et al.*, (2009) designed the controlled release microspheres of Simvastatin, by using ethyl cellulose polymer. Simvastatin-Ethylcellulose microspheres were prepared by water-in-oil-in-oil double emulsion solvent diffusion method and evaluated for entrapment efficiency, *in-vitro* drug release behavior, particle size and size distribution. The designed microspheres were spherical, free flowing and size distribution was between 24-48 μm. The entrapment efficiency and percentage yield were 83.67% & 84.31% respectively. The drug release was controlled for 12h.[(67)]

V.M.Pandya *et al., (2011)* formulated and evaluated Nanosuspension of Simvastatin and studied the effect of different stabilizer on the Simvastatin Nanosuspension. Prepared nanosuspensions was evaluated for its particle size study, *in-vitro* dissolution study and characterized by Screening Electron Microscopy (SEM). Nanosuspension prepared with the PVPK-30 has improved dissolution rate as compare to all other stabilizer because of decreases in particle size (417nm) as compared to micro suspension of Simvastatin. These studies indicate the suitability of PVPK-30 as a stabilizer in the formulation of nanosuspension. [(68)]

J Shinde *et al.*, (2011) designed and evaluated Polylactic-co-glycolic acid nanoparticles containing Simvastatin. Nanoparticles were prepared by precipitation-solvent deposition method using 3^2 full factorial design. *In-vitro* drug release study of selected factorial formulations (PS1, PS4, PS7) showed, 84.56%, 89.65 % and 73.46 % release respectively in 24 hrs. From the results indicated that simvastatin loaded PLGA nanoparticles have potent effective in sustaining drug release for a prolonged period. [(69)]

Pankaj *et al.*,(2012) developed bi-layer tablets of Simvastatin using hydrophilic and or hydrophobic polymers by wet granulation method using hydrophilic and or hydrophobic polymers. From the formulated uncoated tablet of Simvastatin is evaluated successfully within the evaluation parameters which suggest that the tablet have better therapeutic level in systematic circulation.[(70)]

P.C.Chang *et al.*,(2012) developed controlled-release microsphere formulation by encapsulating platelet-derived growth factor (PDGF) and simvastatin through coaxial electrohydrodynamic atomization. About 10% PDLLA (Poly-D,L-lactide (PDLLA, Mw: 24,300–75,000) and 10% PLGA respectively used as polymer to serve as core and shell matrix. The study revealed that PLGA (PDLLA) microspheres are capable of controlling release of PDGF-simvastatin mimicking in vivo-specific situation with an improved biocompatible and bioactive profile. (71)

Y. M. Jagtap *et al.*, (2012) studied the floating microspheres of simvastatin were prepared by emulsion solvent evaporation technique by employed with different polymers such as ethyl cellulose, Eudragit® RS and Eudragit® RL. Release modifiers studied were HPMC K4M, HPMC E50 LV and Eudragit® EPO. Ethyl cellulose and Eudragit® EPO resulted microspheres with high percentage yield, extreme spherical shape. Ethyl cellulose microspheres fabricated by using HPMC K4M exhibit more sustained drug release than the microspheres formulated with the HPMC E50 LV. Amid these polymers HPMC E50 LV showed good balance an optimism and the drug release. (72)

Parmar *et al.(2012)* studied to increase the solubility of poorly water-soluble drug of Simvastatin, by the formation of solid dispersion and complex and using the microwave induction technique on these formations. For solid dispersion method dispersion carrier used were poloxamer 407 and gelucire 44/14. The fusion method was used to prepare the dispersions. In the solid dispersion technique, Simvastatin show higher increase in solubility with gelucire 44/14 in the ratio of 1:5 as compare to poloxamer 407. In fusion method simvastatin show higher solubility with simvastatin and gelucire 44/14 after 10 mins time interval as compare to poloxamer 407 and β-cyclodextrin. By using gelucire 44/14 with Simvastatin it show 94% increase in solubility of Simvastatin as compare to pure drug in water. (73)

B. Brahmaiah *et al.*, (2013) formulated mucoadhesive microspheres of Simvastatin microspheres were prepared by orifice-ionotropic gelation method using polymers such as HPMC (K 100 M), Carbopol 940P, sodium CMC, guar gum, sodium alginate, ethyl cellulose, methyl cellulose and xanthan gum. The microspheres were characterized for drug content, entrapment efficiency, mucoadhesive property by *in-vitro* wash-off test and *in-vitro* drug release. The formulation F10 was selected as an ideal formulation based on the *in-vitro* release profile which shows an extended drug release of 97.11% upto 8 hrs in phosphate buffer of pH 7.0. (74)

S.Rao *et al.*, (2014) developed mucoadhesive microsphere of simvastatin by utilization of various polymers such as HPMC K100M, Sodium Ethyl cellulose, Methyl cellulose, Guar gum, Carbopol 940, sodium alginate and Xanthum gum by ionic gelation method. Simvastatin release from sodium alginate: methyl cellulose reported as slow and extended over a period of 08 hrs and the microspheres feasible for oral controlled release formulation. (75)

B. M. Alam *et al.*, (2014) prepared a series by grafting of simvastatin to PMMA (Poly methacrylic acid) in an esterification reaction. Then the resultant crosslinked with ethylene glycol to generate PMMA-simvastatin hydrogel. Simvastatin released at pH 7 (intestine media) and the material capable to release a higher rate (59.00±1.06 %) uniformly with a rate of (1.11±0.02 %) per hr. during 53 hr. of the release process. The formulation shown the potential to release uniformly of maximum 9.99±0.09% of simvastatin at stomach pH 1.0 (acidic medium) during the first 3 hr. period. (76)

R. Masaeli *et al.*,(2016) fabricated simvastatin-loaded poly(lactic-co-glycolic acid) (Simvastatin loaded PLGA)microspheres + CPC composite (simvastatin loaded PLGA+nanostrontium-CPC) via oil-in-water (O/W) emulsion/solvent evaporation method. (77)

R.Parhi *et al.*,(2016) formulated matrix type of simvastatin transdermal film with poly(vinyl alcohol) (PVA) and eudragit RL100 (EG) and dibutyl phthalate (DBT). Composition of optimized film was found to be 2 % of SS, 2:1 ratio of PVA:EG & 40% of DBT, under these conditions, the SSTF exhibited a predicted value of tensile strength and flux of 11.871 MPa and 43.569 μg/cm2/h, respectively. The formulation shown absence of skin irritation ,hence the film reported as safe and well tolerated for transdermal formulation. (78)

N. Selvasudha *et al.*, (2017) reported design and evaluation of Nano-formulations of Simvastatin using different polymers (Chitosan, Guar gum, almond gum) by solvent evaporation method for anti- hyperlipidemic activity. Preliminary studies supported for chitosan formulations due to the expected particle size (543 ± 26 nm) among the three polymer formulations. Formation of low molecular weight chitosan 70,000 ± 10,000 Da maximized swelling & mucoadhesive properties. It shown better absorption at intestine prolonged the drug release up to 66.18 ± 1.26% in SIF during the *in-vitro* study. (79)

2.4.0 LITERATURE RELATED TO ANIMAL STUDIES

Paul A. Lapchak *et al.*, (2009) demonstrated the pharmacological effects of simvastatin administered alone and in combination with tissue plasminogen activator (tPA) to estimate ischemia and hemorrhage in large clot embolized New Zealand white rabbits. Simvastatin (20 mg/kg, SC in DMSO) was administered 24 hr. and 4 h prior to large clot embolization in order to achieve a "loading dose" pretreatment with the drug. In combination study tissue Plasminogen Activator (tPA) (3.3 mg/kg, IV) administered 1.0 hr. following embolization. Intravenous tPA administration markedly increased hemorrhage volume by 175.0% ($p=0.015$) and hemorrhage incidence by 60% ($p>0.05$) compared to control. Simvastatin treatment significantly decreased tPA-induced hemorrhage incidence ($p=0.022$) and volume ($p=0.0001$) following embolization. [80]

Y. Naito *et al.*, (2013) studied the development of Simvastatin loaded PLGA microspheres via O/W emulsion solvent evaporation method with adequate morphologic characteristics and high encapsulation efficiency for incorporation in bone cements. The biodegradable characteristic of the microspheres shown a slow release and the duration of the release lasted for more than 01 month. The *in-vitro* release profile of the simvastatin from the microsphere during the first 24 hr. of incubation approximately about 0.15 mg subsequently, the statin release rate was approximately 0.015 mg/day. The cumulative release amount at day 31 was 0.583 mg. The in vivo study revealed that the microspheres containing simvastatin markedly enhanced bone formation in the rabbit calvaria critical size defect. [81]

G. Kisvári *et al.*, (2015) studied PI 3-Kinase / Akt pathway is involved in the activation of endothelial nitric oxide synthase (eNOS) and in the subsequent increase of nitric oxide (NO) production of acute simvastatin treated anesthetized dogs, subjected to 25 min occlusion and reperfusion of the left anterior descending coronary artery. Utilizing the same model, 12 dogs received controls given the solvent of simvastatin and 11 dogs treated with intracoronary administer of simvastatin (0.1 mg/kg) were received wortmannin (1.5 mg/kg ic.), a selective inhibitor of PI3-kinase. Rest of 13 dogs the effects of DMSO (0.1%), the vehicle of wortmannin, were examined. Study concluded with NO-dependent anti-arrhythmic effect of simvastatin involves the rapid activation of eNOS through the stimulation of the PI3-kinase/Akt pathway. [82]

P. Zheng *et al.*, (2017) explored an ameliorates graft-vs-host disease by regulating angiopoietin-1 and angiopoietin-2 in a murine model. By *in-vitro* simvastatin administration increased Ang-1 production and release but contrary inhibited Ang-2 release from EA.hy926 ECs. Simvastatin enhances the survival of a GVHD mice, attenuated the histo-pathological GVHD grades and plasma levels of Ang-2, and increased the plasma levels of Ang-1 as well as the aortic endothelial levels of Ang-1and Ang-2. [83]

2.5.0 LITERATURE RELATED TO CLINICAL STUDIES

P.Kniff *et al.*, (1990) investigated. a group of 120 patients with heterozygous familial hypercholesterolemia (FH) the influence of the apolipoprotein E (apoE) polymorphism on pre-treatment plasma lipid levels and on the response to treatment with simvastatin was studied. After 12 weeks use of a daily dose of 40 mg simvastatin, the plasma total cholesterol, low density lipoprotein (LDL)-cholesterol and plasma triglyceride levels were reduced on average by 338, 38% and 19% respectively. At the same time high density lipoprotein (HDL)-cholesterol concentration increased on average by 7%. [84]

B.Leung et.at.,(2003) demonstrated.3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) exert favorable effects on lipoprotein metabolism but may also possess anti-inflammatory properties. From the ex-vivo analysis reported that T cell contact-activated macrophages was suppressed by simvastatin, suggesting that such observations have direct clinical relevance. and illustrated therapeutic potential of statin-sensitive pathways in inflammatory arthritis. [85]

T.Prueksaritanont *et al.,(2003)* identified the cytochrome P450 (CYP) isoforms responsible for the metabolism of simvastatin hydroxy acid (SVA), the most potent metabolite of simvastatin (SV). The metabolism of SVA in human liver microsomes is catalyzed primarily (≥ 80%) by CYP3A4/5, with a minor contribution from CYP2C8.CYP2D6 and other major CYP isoforms are not involved in the hepatic metabolism of SVA. [86]

Ruth Penn MD *et al.*,(2005) Studied the effect of administration of single dose of atorvastatin, simvastatin, or extended-release niacin on the pharmacokinetics and safety of a single dose of fenofibrate Insoluble Drug Delivery®-Microparticle (IDD-P). studies conducted an open-label, single-center, randomized, 4-treatment, 4-period crossover study in healthy adult volunteers-IDDP fenofibrate 160mg tablet alone; IDD-P fenofibrate160mg tablet + atorvastatin 10mg tablet; IDD-P fenofibrate 160mg tablet +

simvastatin 10mg tablet; and IDD-P fenofibrate 160mg tablet+ ER niacin 500mg tablet. Furthermore, a single dose of IDD-P fenofibrate, administered alone or in combination with other lipid-lowering agents is normally well tolerated. (87)

Neil A. Turner *et al.,(2005)* studied the increased matrix metalloproteinase-9 (MMP-9) expression is associated with intimal hyperplasia in saphenous vein (SV) bypass grafts. Recent evidence suggests that HMG-CoA reductase inhibitors (statins) can prevent the progression of vein graft failure. The data suggest that simvastatin reduces MMP-9 secretion from human SV-SMC by inhibiting the RhoA/ROCK pathway and decreasing MMP-9 mRNA levels independently of effects on signaling pathways required for MMP-9 gene expression. (88)

D. Haas *et al.,(2007)* studied the Smith–Lemli–Opitz syndrome (SLOS) a malformation syndrome caused by deficiency of 7- dehydrocholesterol reductase catalyzing the last step of cholesterol biosynthesis. This results in an accumulation of 7 & 8 dehydrocholesterol (7+8–DHC) and, in most patients, a deficiency of cholesterol. Whereas it was due to an increasing cholesterol concentration in the cholesterol-only cohort, a decreasing 7+8–DHC concentration was demonstrated in the cohort receiving additional simvastatin. (89)

Hamed Vaziri *et al., (2007)* evaluated the effect of simvastatin on ligature-induced bone resorption in the mandible of the ovariectomized rat. From study, it is concluded that simvastatin shows protective features against the impact of periodontitis on attachment apparatus and alveolar bone. (90)

John R. Guyton *et al.,*(2008) evaluated the safety and lipid altering potency of ezetimibe/simvastatin (E/S) Co-administration with extended release niacin (N) in patients with type IIa or IIb hyperlipidemia. This study about 24-week multicenter, randomized, double-blind study, type IIa or IIb hyperlipidemic patients were randomized for treatment. Combination treatment with E/S plus N showed superior lipid-altering efficacy compared with N or E/S in type IIa or IIb hyperlipidemia patients and was generally well tolerated aside from N-associated flushing. (91)

A.Pradeep *et al.,(2010)* Studied clinical effect of sub gingivally delivered simvastatin in the treatment of patients with chronic periodontitis: a randomized clinical trial. Various drugs have been studied using local delivery to improve the periodontal health and to

achieve periodontal regeneration treated with locally delivered SMV in patients with chronic periodontitis. [92]

Elewa *et al.,(2010)* studied different angiogenic modulating targets and performed extensive investigation both experimentally and clinically from both animal and human studies regarding the effects of statins on angiogenesis in ischemic heart disease, stroke, ocular diseases, and cancer. The study of statins reported as safe, orally available agents that acquire novel therapeutic indications through their angiogenic modulating effects. [93]

Jang.S *et al.,(2010)*compared the controlled-release (CR) formulation of simvastatin. The goal of this study was to compare the pharmacokinetics of the new CR formulation and an IR formulation of simvastatin after single- and multiple-dose administration in healthy Korean subjects. The simvastatin CR and IR formulations were well tolerated, with no serious AEs observed. [94]

Javeer.S *et al.*,(2013) studied the low viscosity grade hydroxypropyl methyl cellulose (Methocel _ E3 LV and Methocel_ E5 LV) to enhance the solubility and dissolution of poorly water-soluble drug simvastatin (SIM). Two different technologies, hot melt extrusion and spray drying were employed. Results of the study shown the conversion of crystalline form drug into amorphous form indicating increase in dissolution rate and solubility of Simvastatin. [95]

P.chang *et al.*,(2013) investigated the combination and sequential-release of platelet-derived growth factor (PDGF, mitogen) and simvastatin facilitated periodontal regeneration. PDGF and simvastatin were encapsulated in double-walled poly-(D,L-lactide) and poly-(D,L-lactide-co-glycolide) (PDLLA-PLGA) microspheres using the co-axial electrohydrodynamic atomization technique. [96]

Michael Chorev *et al., (2013)* studied that Statins, potent compounds that inhibit cholesterol synthesis in the liver have been reported to induce bone formation, both in tissue culture and in rats and mice. While PTH demonstrated the expected anabolic effect on bone, SVS failed to stimulate bone formation, despite our verification by LC/MS of the active SVS-OH metabolite in mouse serum. [97]

G.Chong *et al.,(2018)* Studied the Effect of spleen-invigorating, Qi-replenishing and blood-arresting formula on zebrafish models with simvastatin-induced hemorrhage caused by spleen failing to control blood, in terms of theory of Traditional Chinese Medicine. Outcome of the investigation suggested the Spleen-invigorating, Qi-replenishing and Blood-arresting Formula can reduce the heart hemorrhage ratio of zebrafish induced by simvastatin and increase the Improvement ratio of hemorrhage. [98]

Yong Hoon Kim *et al.,(2019)* Compared statin with ACE inhibitor or ARB therapy in STEMI patients who underwent successful PCI with drug-eluting statins. Studies of the comparative clinical outcomes between statin with angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) in ST-segment elevation myocardial infarction(STEMI) patients are limited. The combination of statin with ACEI may be the preferred.[99]

Mojtaba Dolatshahi *et al.,(2020)* studied the Pharmacological evidence for the involvement of the opioid system in the antidepressant-like effect of simvastatin in mice: study investigated the potential antidepressant-like activity of simvastatin and the possible involvement of opioid systems in the mouse forced swimming test (FST).In conclusion, these findings demonstrated that simvastatin elicited antidepressant-like action possibly through the stimulation of opioidergic pathways, without inducing tolerance and withdrawal signs. [100]

RATIONALE OF CRDDS DESIGN

Designing of CRDDS formulation is a science of importance with special significance on practical aspects of design and manufacturing. The pharmaceutical perception regulates CRDDS mainly pivot on diffusion and dissolution mechanism of drug release. Different attempts are reported to retard the release from such systems by using combinations of different agents or additives. Several approaches have been discovered to execute the target of controlling release of a drug. Incorporation of various additives (ingredients) and polymeric material utilized for forming the film materials of biodegradable and non-biodegradable polymer material (design consideration). For developing a Controlled Drug Delivery System is the extent and rate of absorption of the drug are very important concerns. Drugs with low rate of absorption shows poor bioavailability, that category of drugs are suitable to formulate into controlled release drug delivery. The drug candidates with more rapid absorption than release promise a successful controlled release product formulation.

Absorption window is another major concern that influences the bioavailability of orally administered drugs and can be obstruct to the develop controlled release drug delivery system. The release of the dosage form is a rate-limiting step in case of Controlled release drug delivery preferably than absorption, hence rapid drug absorption is need from a dosage form in terms of both extent and rate of drug absorption especially for orally administrated drugs. The drug distribution in the body is an essential concern to derive the overall elimination kinetics of the drug. The distribution has close relationship with drug binding to tissue and protein in blood stream. Capacity of high binding of the drug shows the prolonged release. For designing of the CRDDS drug product, drug disposition should be decided based on pharmacokinetic parameters like the volume of distribution (Vd).

3.1.0 Dose & Dosage form of Simvastatin

Tablets -10 mg , 20mg, 40 mg & 80mg

Oral suspension – 20mg/5mL & 40mg/5mL

Extended release tablets (ER)- 20mg & 40 mg

3.1.1 **Rationale of formulation ingredients**

Ingredients	Category	Rationale
Simvastatin	Active ingredient	The mentioned ingredient is main component of the drug product.
Sodium Alginate	Thickening agent	To thicken and harden the product.
Hydroxy propyl methyl cellulose	Semi synthetic polymer	To increase the drug release of formulation over period
Ethyl cellulose	Semi synthetic polymer	To increase the drug release of formulation over period
Carbopol 940	Synthetic polymer (Poly acrylic acid polymer)	To increase the viscosity (gelling mechanism) of formulation for retard the drug release.
PLGA [poly (D,L-lactic -co-glycolide)	Biodegradable polymer	PLGA polymer is falls under the category of biodegradable and promising ideal sustained release.
Polyvinyl alcohol	Adhesive cum emulsifier	To emulsify and sizing of formulation

3.2.0 Rationale of Drug product formulation

To develop the CRDDS formulation have various parameters such as disease category, Potency of drug, drug permeability, dosing frequency and toxicological profile of drug selection are considered during the formulation development.
According to the medical expenditure panel survey, Simvastatin is the most prescribed drug from 2012-2013 with the overall percentage of 41.4% among the statin category drugs. Currently there is no commercial product of simvastatin CR formulation, since simvastatin was selected for the CR formulation developmental study. [101]

3.3.0 Objective of the study

1. To develop an extended release product of poorly water-soluble simvastatin drug with suitable additives.
2. To develop a dissolution control type formulation, enhance the product stability during storage period.
3. To formulate and optimize Extended release microsphere formulation and formulae.
4. To perform *in-vitro* release study of prepared microsphere formulation.
5. Drug product performance evaluation (characterization study).
6. Assessment of drug product efficacy by “In-vitro” methods.

PLAN OF STUDY

Aim

The current research work was carried out to formulate and evaluate the long acting microsphere formulation of Simvastatin drug.

Phases of study

The study is performed as per following segments: -

1. Literature survey through Books, journals and Web sources.
2. Procurement of API, Excipients, polymers and solvents.
3. Pre-formulation studies.
4. Formulation developmental studies.
5. Characterization studies of formulated microspheres.
6. Stability studies.

ATHEROSCLEROSIS

Atherosclerosis is a disease condition in which plaque construct inside the arteries. Arteries especially the Plaques are made up of fat, cholesterol, calcium, and other substances found in the blood. Atherosclerosis is considered the major cause of cardiovascular diseases. Atherosclerotic cardiovascular disease mainly involving in heart and brain: ischemic heart disease (IHD) and ischemic stroke. Atherosclerosis is a predominantly asymptomatic condition; it is difficult to determine the incidence accurately. IHD and stroke are the world's first and fifth causes of death respectively.[102,103]

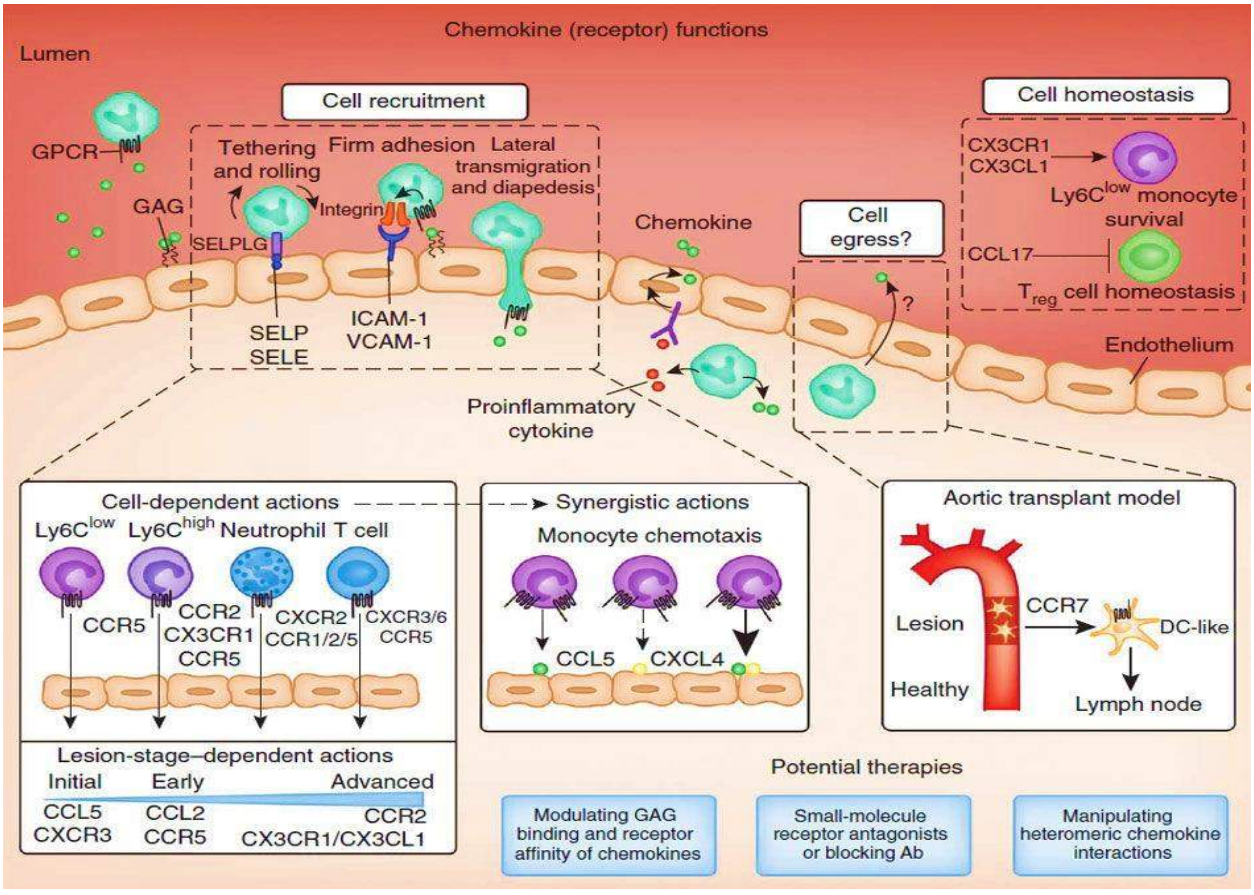


Figure 13: Pathophysiology of Atherosclerosis

PREVALENCE

In USA annually an about 610,000 people die of heart disease Coronary heart disease is the leading cause of death in the Western countries killing over 370,000 people annually. On an average, about 735,000 Americans have a heart attack every year. Out of the trend 525,000 have an initial attack, and 210,000 have a recurrent attack. This higher prevalence of atherosclerosis. It is reported that nearly 795,000 people suffer from stroke every year in the US resulting in about 140,323 deaths. The major form of stroke, ischemic stroke is due to ASCVD. It has been reported that 75% of acute myocardial infarctions occur from plaque rupture.

Cardiovascular diseases, especially coronary heart disease (CHD), are epidemic in India. [104]The Registrar General of India reported that CHD led to 17.0% of total deaths and 26.0% of adult deaths in 2001-2003, which markedly raised to 23.0 % of total and 32.0% of adult deaths in 2010-2013. The World Health Organization (WHO) and Global Burden of Disease Study also have highlighted increasing trends in years of life lost and disability-adjusted life years from CHD in India. In India, studies have reported enhancing coronary heart disease prevalence over the last 60 years, from 1.0% to 9%-10% in urban populations and <1% to 4%-6% in rural populations.

Many epidemiologic studies in North America and Europe have recognized numerous risk factors for the development and progression of atherosclerosis. They may facilitate atherosclerosis through their effects on low-density lipoprotein (LDL) particles and inflammation. [105,106]

SIGNS

The initial sign of atherosclerosis can begin to develop during adolescence, with streaks of white blood cells appearing on the artery wall.

SYMPTOMS

The patient feels and describes, such as pain and rashes. The symptoms of the disease depend on which arteries are affected:

Type of arteries	Functions	Symptoms
Carotid Arteries	These arteries provide blood to the brain, when the blood supply is limited patients can suffer stroke and may experience	• Weakness • Difficulty breathing • Headache • Facial numbness • Paralysis
Coronary Arteries	These arteries provide blood to the heart, when the blood supply to the heart is limited it can cause angina and heart attack.	• Vomiting • Extreme anxiety • Chest pain • Coughing • Feeling faint
Renal Arteries	These artery supply blood to the kidneys; if the blood supply becomes limited, there is a serious risk of developing chronic kidney disease.	• Loss of appetite Swelling of the hands and feet • Difficulty concentrating
Peripheral artery	These arteries are supplying the blood to the limbs, usually the legs.	• Leg pain • Pain in calve & thighs /hips

Causes of Atherosclerosis

The condition is caused by macrophage white blood cells and fat that accumulates in arteries -the white blood cells are originally sent by the body's immune system to clean up LDL cholesterol pockets. When they affixed to an artery, they secrete a molecule called netrin-1,this stops normal migration of the macrophages out of the arteries. As a result, the left mixture of clumped up cholesterol pockets and white blood cells, this is the plaque that can disrupt blood flow.

Certain factors that can damage the inner area of the artery (endothelium) and can trigger atherosclerosis includes:

- High Blood Pressure
- High levels of cholesterol
- Smoking

- High levels of sugar in the blood

Areas of the artery that are damaged are likely to have plaque build-up which can eventually break open. When the plaque breaks open, blood cell fragments called thrombocytes (or platelets) accumulate at the affected area. These fragments can then stick together, forming blood clots.

Diabetes: Diabetic patients with poorly controlled diabetes, who frequently have excess blood glucose levels, are much more likely to develop atherosclerosis.

Genetics : People with a parent or sibling who has/had atherosclerosis and cardiovascular disease have a much higher risk of developing atherosclerosis than others.

Management

Lifestyle modification : The changes will focus on weight management, physical activity and a healthy diet. To recommend eating foods high in soluble fiber and limited intake of saturated fats, sodium and alcohol.

Medication: consumption of medicine will prevent the build-up of plaque or to help prevent blood clots (antiplatelet). Other medications such as calcium channel blockers, fibrates, antiplatelet drug and statins may be prescribed to lower cholesterol and Angiotensin-converting enzyme (ACE) inhibitors to lower blood pressure.

Surgery : Severe cases of atherosclerosis may be treated by surgical procedures, such as angioplasty or coronary artery bypass grafting (CABG).Angioplasty involves expanding the artery and opening the blockage, so that the blood can flow through properly again. CABG is another form of surgery that can improve blood flow to the heart by using arteries from other parts of the body to bypass a narrowed coronary artery.

Exercise : Exercise will improve fitness level and lower blood pressure. For obese patients, exercise can help to lose weight.

HYPERLIPIDEMIA

Hyperlipidemia means high levels of lipids (fat) in the blood, which may be physiological postprandially. Hyperlipidemia results from unbalanced metabolism of the lipoproteins with either excess production, altered clearance, or both. These resulted from disturbances of lipid metabolism and shown elevated serum concentrations of nonpolar fats (i.e., triglyceride (TG) and cholesterol esters (CEs).It is most commonly multifactorial, involving both environmental and intrinsic factors.

Many researches recognized between a mild elevation in serum triglycerides (up to 500 mg%) and a great elevation (> 500 mg%; lipemia or hyperlipemia).

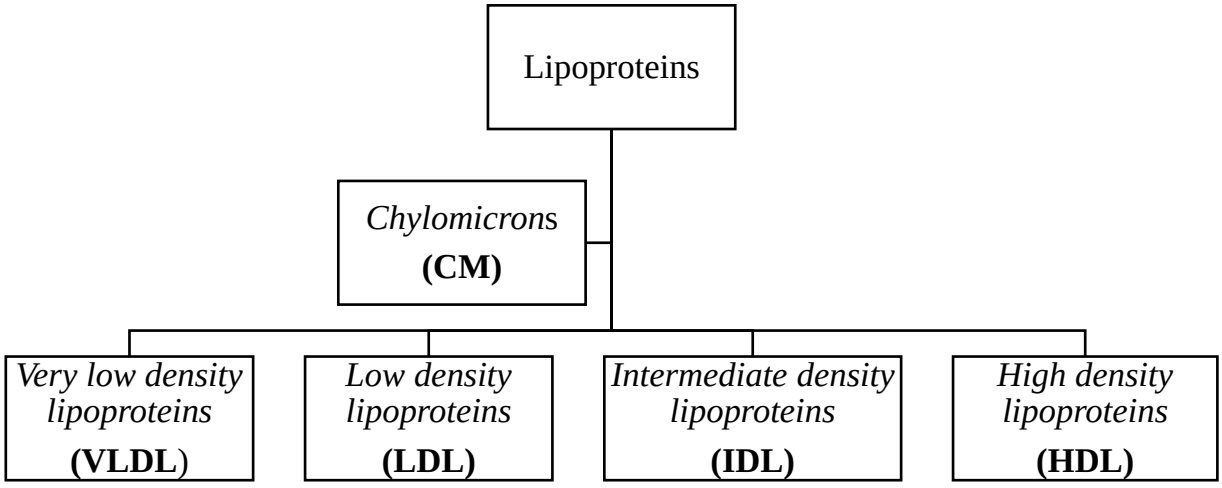


Figure 14: Classification of Lipoproteins

HYPERLIPIDEMIA CLASSIFICATION

Hyperlipidemia broadly classed into two major categories

1. Primary Hyperlipidemia
2. Secondary Hyperlipidemia [107]

Primary: it is also called familial due to a genetic defect; it may be monogenic: a single gene defect or polygenic: multiple gene defects. Primary hyperlipidemia can usually be

resolved in tone of the abnormal lipoprotein patterns and the condition sub-classed into following categories.

Familial hypercholesterolemia (**FH**)
Familial hypercholesterolemia is a genetic disorder characterized by high cholesterol levels, specifically very high levels of low-density lipoprotein (LDL, "bad cholesterol"), in the blood and early cardiovascular disease.

Polygenic hypercholesterolemia
It is the most common form of familial hyperlipidemia. In that plasma cholesterol is not as high as FH and is influenced by environmental factors such as diet.

Familial combined hyperlipidemia
Familial combined hyperlipidemia results in elevated cholesterol and triglycerides and which is associated with diabetes, obesity, cutaneous demonstration of hyperlipidemia and premature ischemic heart disease (IHD).

Familial chylomicronemia
Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disorder occurred by mutations in lipoprotein lipase consequences in accumulation of chylo - microns in plasma and hypertriglyceridemia. Elevated triglycerides cause several complications in patients, the most serious being episodes of acute pancreatitis.

Familial hypertriglyceridemia
Familial hypertriglyceridemia is an autosomal dominant condition occurring in approx. 1.0% of the total population. Triglyceride levels are increased and as a result of excess hepatic production of VLDL or heterozygous LPL deficiency.

Secondary: it is acquired because it is caused by another disorder like diabetes, nephritic syndrome, chronic alcoholism, hypothyroidism and with use of drugs like corticosteroids, beta blockers and oral contraceptives. Secondary hyperlipidemia together with significant hypertriglyceridemia can cause pancreatitis. Secondary hyperlipidemic caused by consumption of alcohol,anti-reterovirals, thiazides also.

SYMPTOMS

- Hyperlipidemia does not have any stipulated symptoms, but they are generally identified during routine examination or until it behaves the threaten phase of a stroke or heart attack.

- Patients with high blood cholesterol level or patients with the familial forms of the disorder can develop xanthomas which are deposits of cholesterol may form under the skin, especially under the eyes.
- At the same time, patients with elevated levels of triglycerides may develop numerous pimple-like lesions at different sites in their body.

Types of hyperlipidemia (Fredrickson's classification by World Health Organization)

Type of Hyperlipidemia	Triglyceride	Cholesterol	Lipoprotein raised
I	Raised	-	Chylomicron
IIa	Normal	Raised	LDL
IIb	Raised	Raised	LDL +VLDL
III	Raised	Raised	IDL
IV	Raised	Normal or Raised	VLDL
V	Markedly raised	Raised	Chylomicron +VLDL

Cause	Examples
Metabolic and nutritional	Obesity, alcohol
Endocrine	Diabetes mellitus, pregnancy, Hypothyroidism
Drugs	β-blockers, thiazides & Estrogens
Renal disease	Chronic renal failure, Nephrotic syndrome.
Liver disease	Biliary obstruction

Classification of Plasma Lipid Levels (Mg/dL) [108]

Non-HDL-C		LDL-C		Triglycerides		HDL-C	
Range	**Grade**	**Range**	**Grade**	**Range**	**Grade**	**Range**	**Grade**
<130	Desirable	< 70	Optimal	<150	Normal	<40	Low
130-159	Above desirable	< 100	Desirable	150-199	Borderline high		
160-189	Borderline high	100-129	Above desirable	200-499	High		

190-219	High	130-159	Borderline high	≥ 500	Very high	>60	High (Desirable)
≥ 220	Very high	160-189	High	-	-	-	-
-	-	≥ 190	Very high	-	-	-	-

Mechanism of statins

Statins exhibits their major action in reduction of LDL levels through mevalonic acid like moiety that competitively inhibits HMG-CoA reductase. By reducing the conversion of HMG-CoA to mevalonate, statins inhibit rate-limiting step in cholesterol biosynthesis. Statins affect blood cholesterol levels by inhibiting hepatic cholesterol synthesis, which results in increased expression of the LDL receptor gene. The reduction in hepatic VLDL production induced by statins is thought to be mediated by reduced synthesis of cholesterol, a required component of VLDLs. [109]

Hyperlipemia screening

The condition can be screened with the patients fasting profile such as TG,HDL,TC, LDL-C. It is preferably recommended an adult have age more than 20 years and should be repeated for at every frequency of year for early detection. [110]

Management

The main objective of hyperlipidemia management is to maintain blood cholesterol level within the normal range as much as possible. The following factors can reduce the risk and helpful for manage the lipidemic level in control.

- Lifestyle modification focusing on the reduction of saturated fat and cholesterol intake
- Weight loss
- Increased physical activity
- Smoking cessation

Pharmacotherapy

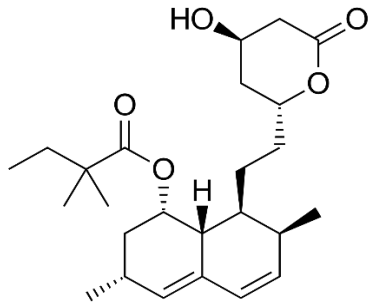
The primary drug choices for lipid lowering therapy is administration of lipid lowering drugs such as statins, fibrate, bile acid sequestering agent derivatives.

- HMG CoA reductase inhibitor(statins)
- Fibrates(gemfibrozil, clofibrate, fenofibrate)
- Niacin(nicotinic acid)
- Bile acid binding resins (colestipol, Cholestyramine).

DRUG PROFILE

SIMVASTATIN

Structure [111]



Physical description : Solid

Description: White to off-white crystalline powder

IUPAC Name: [(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2 yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethyl butanoate

Molecular formula: $C_{25}H_{38}O_5$

Molecular Weight : 418.6 g/mol

Melting point : 135-138 °C

Solubility: chloroform:610mg/mL, DMSO:540mg/mL, methanol: 200mg/mL, ethanol:160mg/mL, n-hexane: 0.15mg/mL, 0.1 M HCl :0.06 mg/mL; propylene glycol: 30mg/mL and 0.1 M NaOH :70mg/mL

Octanol/Water partition coefficient : 4.68

Hygroscopicity : Non-Hygroscopic

Storage conditions: Should be stored in well-closed containers at 20-25 °C

Therapeutic category : Antilipemic Agents

Pharmacological Classification

Anticholesteremic Agents: Substances used to decrease plasma cholesterol levels.

Hydroxy methyl glutaryl-CoA Reductase Inhibitors: Compounds that inhibit HMG-CoA reductases. They have been shown to directly inhibits cholesterol synthesis.

Hypolipidemic Agents: Substances that lower the levels of certain lipids in the blood .They are mainly employed in th treatment of Hyperlipidemia's.

Pharmacological action

Mechanism of Action

Simvastatin is a prodrug and is hydrolyzed to its active β-hydroxy acid form and simvastatin acid after administration. Simvastatin is a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the biosynthetic pathway for cholesterol. In addition, simvastatin reduces VLDL and TG and increases HDL-C.

Pharmacokinetic profile

Absorption

Simvastatin drug estimated closer to an intravenous reference dose in each of two animal species examined, averaged about 85.0% of an oral dose. After oral dosing simvastatin attained significant higher concentration in the liver than non-target tissues. Simvastatin undergoes substantial first pass metabolism, hence the availability of drug in the systemic is low. Peak plasma concentration happens after 1.3 to 2.4 hrs of drug administration.

Distribution

Simvastatin and their metabolites (β-Hydroxy acid) are highly capable to bind with human plasma proteins. The estimated value is about approximately 95.0%. Simvastatin can cross the blood-brain-barrier. simvastatin undergoes extensive first-pass extraction in the liver, the availability of the drug to the general circulation is low (<5.0 %).

Metabolism

Hepatic, simvastatin is a substrate for CYP3A4. The major active metabolites of simvastatin are β-hydroxy acid metabolite and its 6'-hydroxy, 6'-hydroxymethyl, and 6'-exomethylene derivatives.

Excretion
Simvastatin elimination studies in human exhibits (14C-labeled), 13% of the dose excreted via urine and 60% via feces. Biological half-life (t1/2) reported as 3.0 hrs.

Dose
5-80 mg orally once daily

Dosage form
Tablet-Oral 5mg, 10mg, 20mg, 40mg, 80mg.

Adverse drug effects

- Rhabdomyolysis with myoglobinuria and acute renal failure and myopathy
- (including myolysis).
- Upper respiratory infection
- Headache
- Myalgia
- Abdominal pain
- Constipation and Nausea

Indication and use
Simvastatin used as an adjunct to dietary therapy to treat primary hypercholesterolemia (heterozygous familial and nonfamilial) mixed dyslipidemia and hypertriglyceridemia and prescribed to reduce the risk of CHD mortality and cardiovascular events. Simvastatin indicated for homozygous familial hypercholesterolemia as an adjunct to other lipid lowering therapies or when other such therapies are not available.

Overdose
There is no specific treatment in the event of overdose. In the event of overdose, the patient should be treated symptomatically, and supportive measures required. Hemodialysis does not significantly enhance clearance of clearance of Simvastatin.

Warnings and precautions
Risks increase with highest doses and concomitant use of certain medicines like cyclosporine and Danazol. Predisposing factors include advanced age (≥ 65) , female gender, uncontrolled hypothyroidism and renal impairment. Simvastatin therapy should be discontinued immediately if myopathy is diagnosed or suspected.
Persistent elevations in hepatic transaminases can occur. Monitor liver enzymes before and during treatment.

EXCIPIENTS PROFILE

HYDROXYPROPYL METHYLCELLULOSE

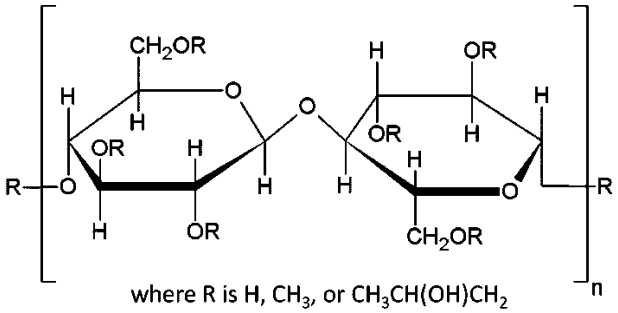
Synonyms [112]

Benecel MHPC; E464; hydroxypropyl methylcellulose;
HPMC; Methocel; methylcellulose propylene glycol ether;
methyl hydroxypropyl cellulose; Metolose; Tylopur.

Nonproprietary Names
BP: Hypromellose
JP: Hydroxypropyl methylcellulose
Ph.Eur: Hypromellosum
USP: Hypromellose

Description : white or creamy white fibrous or granular powder. odorless and tasteless.

Structure



Molecular formula : $C_{20}H_{38}O_{11}$

Molecular Weight :454.5 g/mol

Functional Category : Coating agent, tablet binder, film-former, rate-controlling polymer for sustained release formulation, stabilizing agent, suspending agent; Viscosifier.

Solubility : Soluble in cold water, forming a viscous colloidal solution. Practically insoluble in chloroform, ethanol(95%),& ether, but soluble in mixtures of ethanol & dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol.

Melting point: 190–200°C (Brown colour formation) chars at 225–230°C.

Glass transition temperature :170–180°C.

Viscosity : 3-100000 mPa.s **(**a wide range of viscosity types are commercially available.)

Applications in Pharmaceutical Formulation

- In oral products, Hypromellose is primarily used as a tablet binder in film-coating and as a matrix for use in extended-release tablet formulations
- Hypromellose is also used as a suspending and thickening agent in topical formulations
- Used as an emulsifier, suspending agent, and stabilizing agent in topical gels and ointments.
- Hypromellose is used in the manufacture of capsules, as an adhesive in plastic bandages, and as a wetting agent for hard contact lenses

Acidity/alkalinity: pH : 5.5 to 8.0 in 1% w/w aqueous solution.

Moisture content: Hypromellose absorbs moisture from the atmosphere;

Gel point : 50–90°C

Stability: Hypromellose powder is a stable material, even it is hygroscopic after drying. Solutions are stable at pH 3–11.

Storage Conditions: Hypromellose powder should be stored in a well-closed container, in a cool, dry place.

Incompatibilities: Hypromellose is incompatible with some oxidizing agents.

Safety: Hypromellose is generally nontoxic and nonirritant material, although excessive oral consumption may have a laxative effect.

Regulatory Status: GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide. Included in non-parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

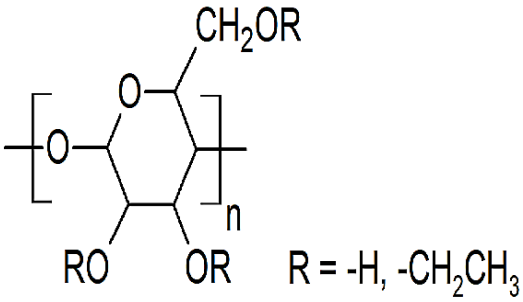
ETHYL CELLULOSE

Synonyms [113]
Aqua coat ECD; Aqualon; E462; Ethocel; Surelease

Nonproprietary Names
BP: Ethylcellulose
Ph. Eur: Ethylcellulose
USPNF: Ethylcellulose

Description: Ethylcellulose is a tasteless, free-flowing, white to light tan colored powder.

Structure:



Molecular formula: $C_{20}H_{38}O_{11}$

Molecular Weight :454.5 g/mol

Functional Category: Coating agent; flavoring fixative; tablet binder; tablet filler; viscosity-increasing agent.

Solubility: Ethyl cellulose is practically insoluble in glycerin, propylene glycol, and water. Ethyl cellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%). Ethyl cellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.

Glass transition temperature:129°–133°C

Density: 0.4 g/cm^3

Viscosity: 7 to 100 mPa s (7–100 cP) at 5% w/v ethyl cellulose dissolved in a solvent blend of 80% toluene :20% ethanol (w/w).

Applications in Pharmaceutical Formulation

- 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 an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films.

Stability: Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis both dilute and concentrated and to salt solutions.

Storage Conditions: Ethyl cellulose should be stored at a temperature not exceeding 32°C (90°F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

Incompatibilities: Incompatible with paraffin wax and microcrystalline wax.

Safety: Ethyl cellulose is combustible. Ethyl cellulose powder may be an irritant to the eyes and eye protection should be worn.

Regulatory Status: GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (oral capsules, suspensions and tablets; topical emulsions and vaginal preparations).

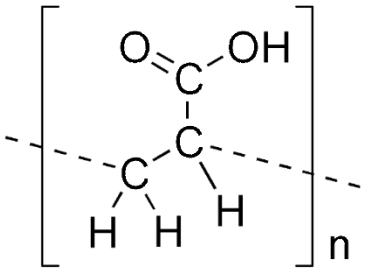
CARBOPOL 940

Synonyms[114]
Acritamer; acrylic acid polymer, Carbopol, carboxy polymethylene, carboxy vinyl polymer; Pemulen; Ultrez.

Nonproprietary Names
BP: Carbomers
USPNF: Carbomer
Ph. Eur: Carbomera

Description
White colored, “fluffy” material, hygroscopic powder with slight characteristic odor.

Structure



Molecular formula: $C_3H_3NaO_2$

Molecular Weight: 94.044489 g/mol

Functional Category

Bio adhesive emulsifier, release-modifying agent; suspending agent; tablet binder; viscosity-increasing agent.

Solubility

Soluble in water and, after neutralization, in ethanol (95%) and glycerin.

Glass transition temperature: 100 -105°C
Melting point: Decomposition occurs within 30 minutes at 260°C

Specific gravity :1.41

Applications in Pharmaceutical Formulation

- Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents.
- Formulations include creams, gels, and ointments for use in ophthalmic, rectal, and topical preparations.
- In tablet formulations, carbomers are used as dry or wet binders and as a rate controlling excipient.
- Carbomer resins have also been investigated in the preparation of sustained-release matrix beads
- Used in oral mucoadhesive controlled drug delivery systems.
- Carbomers are also employed as emulsifying agents in the preparation of oil-in-water emulsions for external use

Stability

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104°C for up to 2 hours without affecting their thickening efficiency. Complete decomposition occurs with heating for 30 minutes at 260°C

Storage Conditions

Carbomer powder should be stored in an airtight, corrosion- resistant container in a cool and dry place. The use of glass, plastic, or resin-lined containers is suggested for the storage of formulations containing carbomer.

Incompatibilities

Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes.

Safety

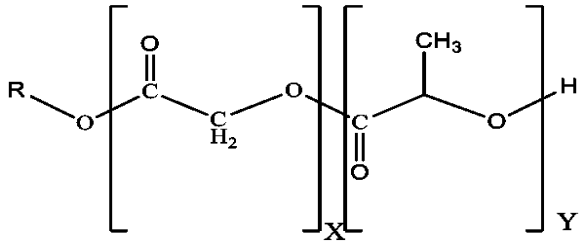
Carbomers are generally nontoxic and nonirritant materials. There is no evidence in humans of hypersensitivity reactions to carbomers used topically.in humans' oral dose of 1-3 gm of carbomer have been used as bulk laxative.

Regulatory Status

Included in the FDA Inactive Ingredients Guide for oral suspensions, tablets; ophthalmic, rectal, and topical preparations transdermal preparations, vaginal suppositories. Included in non-parenteral medicines licensed in Europe.

Poly(lactic-co-glycolic acid)

Synonym[115] : L-PLGA (75 :25)
Structure :



Color : White to light gold

Molecular weight : 40 000–100 000 Da

Inherent viscosity : 0.5–0.8 mPa.s

Description of material : Amorphous nature

Glass transition : 50-55°C

Solubility : Soluble in methylene chloride; tetra hydro furan, ethyl acetate, hexafluoro isopropanol, hexafluoro acetone sesquihydrate and acetone

Specific gravity : 1.30

Tensile strength: 6000–8000 psi

Functional Category : Bioabsorbable; biocompatible; biodegradable material.

Stability and Storage Conditions

The aliphatic polyesters are easily susceptible to hydrolysis in the presence of moisture. Hence, they should be properly stored, preferably refrigerated at below 0°C. It is necessary to allow the polymers to reach room temperature before opening the container.

Safety :

poly(lactide-co-glycolide) is used in parenteral pharmaceutical formulations and are regarded as biodegradable, biocompatible, and bioabsorbable materials. Their biodegradation products are nontoxic, noncarcinogenic, and nonteratogenic. In general, these polyesters exhibit very little hazard.

Regulatory Status

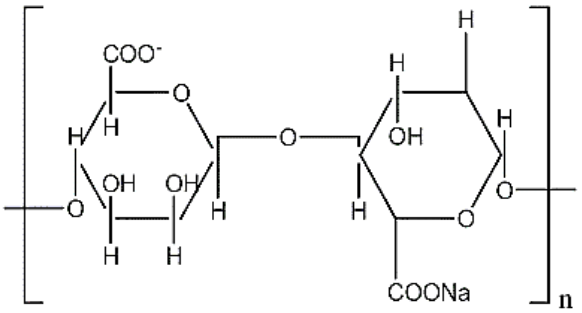
GRAS listed. Included in the Canadian List of Acceptable Nonmedicinal Ingredients.

SODIUM ALGINATE

Nonproprietary Names
BP: Sodium alginate
Ph.Eur: Natrii alginas
USPNF: Sodium alginate

Synonyms : Algin, alginic acid, sodium salt; E401; Kelcosol, Keltone,Protanal,Sodium polymannuronate.

Structure [116]



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

Molecular formula: $C_6H_9NaO_7$

Molecular Weight: 216.12 g/mol

Acidity/alkalinity: pH 7.2 (at 1% w/v aqueous solution).

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

Viscosity (dynamic): 1% w/v aqueous solution, at 20°C,viscosity about 20–400 mPa s (20–400 cP).

Functional Category: Stabilizing agent, suspending agent, tablet and capsule disintegrants, tablet binder; viscosity-increasing agent.
Stability: Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidity and a cool temperature. Aqueous solutions of sodium alginate are most stable at pH 4–10. Below pH 3, alginic acid is precipitated.

Storage : Solutions should not be stored in metal containers. The bulk material should be stored in an airtight container in a cool, dry place.

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

Safety: Sodium alginate is widely used in cosmetics, food products, and pharmaceutical formulations, such as tablets and topical products, including wound dressings. It is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption may be harmful.

Regulatory Status
GRAS listed. Accepted in Europe for use as a food additive. Included in the FDA Inactive Ingredients Guide. Included in non-parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Nonmedicinal Ingredients.

MATERIALS AND METHODS

Ingredients utilized for formulation

S.No	Material name	Source/Supplier
01	Simvastatin	Biocon Ltd, Bangalore
02	Hydroxy propyl methyl cellulose E15LV	Loba Chemie, Mumbai.
03	Ethyl cellulose	Kemphasol,mumbai
04	Carbopol 940	Loba Chemie, Mumbai.
05	Poly(lactic-co-glycolic acid) [75:25]	Sigma-Aldrich
06	Sodium alginate	Nice chemicals, Bangalore
07	Poly vinyl alcohol	Molychem
08	Ethyl acetate	Merck Ltd
09	Chloroform	Merck Ltd
10	Hydrochloric Acid	S.D. Fine chemicals Pvt limited, Mumbai
11	Methanol	S.D. Fine chemicals Pvt limited, Mumbai

Instruments/Equipment's utilized for formulation

S.No	Instrument name	Make
1	Glassware's	Sigma scientific glass Pvt Ltd
2	Weighing balance	Shimadzu scientific instruments
3	Magnetic stirrer	REMI laboratory instruments
4	Mechanical stirrer	REMI laboratory instruments (model: 2 MLH)
5	Hot air oven	New tech instruments
6	UV-Visible Spectrometer	Lab India Pvt Ltd
7	USP type-II Dissolution apparatus	Lab India Pvt Ltd
8	Ultracentrifuge	Plasto crafts Mumbai
9	Scanning Electron microscope	CAREL ZEISS (Model: EVO 18)
10	Fourier Transform Infra-Red Spectrometer	Perkin Elmer (Model: Spectrum Two)
11	Vaccum dryer	Saga engineering co (model: SO-150)
12	Hot plate	Krishna Pvt ltd

PREFORMULATION STUDIES

Preformulation study is an essential tool for drug development that commences during the initial pharmaceutical development process. These studies are systematically designed to generate comprehensive information related to the physicochemical properties, drug-initial excipients compatibility, develop analytical investigations, and other information to directly or indirectly which support formulation development. Outcomes obtained from the pre-formulation investigations reflect useful groundwork information towards the product formulation attempts.

9.1.0 Role of Preformulation During Product Development

The International Council for Harmonization (ICH) recommends criteria for stability and testing conditions and these guidelines may be implemented in designing a formulation with minimal stability risks. Extreme knowledge about the regulatory, technology utilized, resources are mandatory to the product development, which will rationalize the design for any type of formulation development in each product strategy. Preformulation study involves in the application of pharmaceutical principle to characterize the physiochemical parameters of Drug substance for designing the Ideal drug delivery of drug formulation.
To design and develop the novel drug formulation with effective and stable, compatibility assessment of drug with excipients should be performed by various techniques such as UV spectroscopic studies, FT-IR , Differential scanning colorimetry etc., these techniques are considered for estimation of drug- Excipient compatibility. It is an essential process to choose the right excipient for the formulation of drug product.

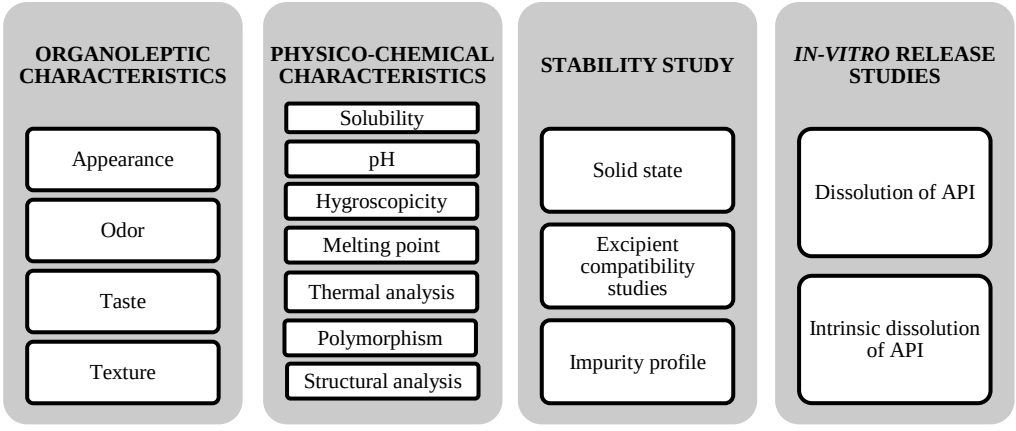


Figure 15: Preformulation developmental study list

COMPATIBILITY STUDY DESIGN

Label code	Ingredients	Container Constrain	Initial values (%)	40°C/75%RH			25°C/ 60%RH
				15 days	30 days	60 days	60 days
SV-01	Simvastatin	OC	99.30	99.28	99.28	99.27	99.29
SV-02	Simvastatin	CC		99.29	99.25	99.24	99.26
SV-03	Simvastatin + HPMC	OC	99.29	98.98	98.53	97.76	99.25
SV-04	Simvastatin + HPMC	CC		99.26	99.10	98.85	99.29
SV-05	Simvastatin + HPMC+Sodium alginate	OC	99.30	99.29	98.56	98.15	98.90
SV-06	Simvastatin + HPMC+Sodium alginate	CC		99.26	99.20	98.55	98.94
SV-07	Simvastatin + Ethyl cellulose	OC	99.30	99.09	98.36	97.95	99.22
SV-08	Simvastatin+Ethyl cellulose	CC		99.29	99.23	99.17	99.29
SV-09	Simvastatin + Ethyl cellulose+Sodium alginate	OC	99.30	99.24	98.51	98.10	99.00
SV-10	Simvastatin + Ethyl cellulose+Sodium alginate	CC		99.28	99.23	99.11	99.24
SV-11	Simvastatin + Carbopol 940	OC	99.30	99.29	99.02	99.05	98.56
SV-12	Simvastatin + Carbopol 940	CC		99.26	99.29	98.78	99.22
SV-13	Simvastatin + Carbopol 940+Sodium alginate	OC	99.30	99.23	99.12	98.95	98.38
SV-14	Simvastatin + Carbopol 940+Sodium alginate	CC		99.26	99.29	98.76	99.22
SV-15	Simvastatin + poly(lactide-co-glycolide)	OC	99.30	99.26	99.03	98.66	98.38
SV-16	Simvastatin + poly(lactide-co-glycolide)	CC		99.29	99.21	98.96	99.15
SV-17	Simvastatin + PLGA +Sodium alginate	OC	99.30	99.25	99.12	98.93	99.17
SV-18	Simvastatin + PLGA +Sodium alginate	CC		99.29	99.19	98.98	99.13

** CC- Closed condition : The respective material stored in the closed container.
** OC-Open condition: The respective material exposed to atmospheric condition in an open container.

SPECTROSCOPIC STUDIES

Characterization study of Simvastatin

The infrared spectrum of pure drug substance of simvastatin was recorded, and spectrum analysis was performed. The dry sample of the drug was thoroughly mixed with potassium bromide and pressed into pellets and then directly placed in sample holder and analyzed under FTIR.

Characterization analysis of polymer materials

The infrared spectrum of Hydroxypropyl methyl cellulose, Ethyl cellulose, Carbopol 940, poly lactic and glycolide acid and sodium alginate was recorded and spectral analysis was done to identify the presence of functional groups. The dry sample of polymers was thoroughly mixed with potassium bromide and pressed into pellets and then directly placed in sample holder and analyzed under FTIR. Likewise, IR spectrums of simvastatin and polymers was analyzed.

Determination of λ_{max} by UV spectroscopy

UV spectrum of Simvastatin was accomplished in 0.1 N HCL, and Phosphate buffer pH 6.8.Accurately weighed quantity of simvastatin (10.0 mg) transferred into 100mL volumetric flask and the volume was made with buffer solution to obtain 100 µg/mL. This solution was treated as stock solution (Stock-I) .From the stock solution-I further diluted to achieve 10 µg/mL by using suitable buffer solution. Then the UV spectrum was recorded at absorbance spectra of 238 nm.

Construction of Standard curve:

Preparation of Stock Solution

Standard stock solution of Simvastatin was prepared by dissolving 10mg of Simvastatin (Pure drug) in 10mL of 0.1N HCL to obtain 1mg/mL (or)1000µg/mL. This solution was treated as stock solution (Stock-I).From the stock solution-I withdraw 1mL of solution and further dilute upto 10mL by using 0.1 N HCL to produce the concentration of 100µg/mL (Stock-II).

Preparation of Calibration curve

From the stock solution (Stock-II) 0.2, 0.4, 0.6 , 0.8 , 1.0 mL were withdrawn and taken into a separate 10 mL volumetric flask. The volume was made upto 10mL by using 0.1 N HCL to obtain the concentration of 2,4,6,8,10 µg/mL respectively. With the aid of UV spectroscopy absorbance of solutions were measured at 238 nm to construct the standard curve. This standard curve employed to determine the drug release from the dosage form.

FORMULATION DEVELOPMENTAL STUDY

The ultimate scope of this current research formulation to develop long acting microspheres as controlled drug delivery system, which deliver the drug in prolong period and achieves therapeutic needs in pharmacotherapy aspects.

The microsphere formulation designed to reduce the frequency of drug intake as well as prevent the uncontrolled burst release from the formulation of drug product. Hence the formulation designed to promise the controlled release with the property of double walled or polymer mixture used in the drug product formulation.

The prepared drug: polymer mixture was analyzed for compatibility studies and assay to understand the product stability and strength.

The formulation study performed with the hydrophilic polymers , cross linked polyacrylate polymer, hydrophobic polymers and smart polymers (poly lactic glycolic acid).

In pharmaceutical application sodium alginate exhibits good thickening and stabilizing property. Hence all the formulation decided to impart the sodium alginate to provide the microsphere thickening for controlled release. [117-127]

Carbopol 940 is hydrophilic and cross-linked polyacrylate polymer which shown good suspending,thickening,gelling and release retardant properties in pharmaceutical formulations. Hence the polymer chosen for formulation.[128-138]

PLGA is biodegradable polymer utilized for encapsulation of micro and nano technology in pharmaceutical formulations. This polymer are smart carriers of drug will product long last duration .It found great success due to biocompatibility,biodegration and favorable release kinetics. [139-150]

PREPARATION OF SIMVASTATIN LOADED MICROSPHERES

Simvastatin microspheres was prepared by the principle of solvent evaporation technique.

Preparation of drug phase
In 25 mL borosilicate glass beaker 40 mg of simvastatin drug was transferred and API dissolved in 5 mL of chloroform and allowed to stir for 10 min by using magnetic beads.

Preparation of polymer phase
The calculated and weighed quantity of polymer (different ratios) was transferred into labelled respective glass beaker containing 10mL of solvent solution and allowed to mix for 15 min by using magnetic beads.

Preparation of surfactant solution
Transferred pre-heated water of 100 mL about 65℃ to 250 mL borosilicate glass beaker and added weighed qty (20) of poly vinyl alcohol into beaker. Then allowed to stir for 15 min and brought down the temperature to 25℃.

Preparation of drug-polymer phase
The prepared drug phase slowly added to the polymer phase under mixing to ensure the homogeneity mixing and uniform drug entrapment to the polymer solution.

Mixing of organic phase
To the 0.20% PVA solution added the pre-mixed drug-polymer phase under the mixing speed of 250 RPM at 25℃ Mixing duration allowed to stand for 06 hrs to evaporate the organic solvents bound to the microsphere formulation.

Washing of microspheres
After evaporation of solvents the drug product were precipitated and the same collected cautiously, then subjected for washing with 15% w/v Methanol solution and washed with water twice.

Drying of microspheres
The microsphere product obtained from the stage of post washing, exposed to hot air oven under the temperature of 28±2℃ for 05 hrs. Finally, the microsphere was collected in dried glass beaker and labelled.

Storage of microspheres
Formulated microspheres were preserved and stored at room temperature.Preferrably 20-25℃

FORMULATION COMPONENTS AND FORMULA

Formula code ↓	Formulation components							
	Simvastatin (mg)	HPMC (mg)	Ethyl cellulose (mg)	Carbopol 940 (mg)	PLGA (mg)	Sodium alginate (mg)	Polyvinyl alcohol (%)	Total (mg)
SMVF-01	40	25	-	-	-	135	0.20	200
SMVF-02	40	50	-	-	-	110	0.20	200
SMVF-03	40	75	-	-	-	85	0.20	200
SMVF-04	40	100	-	-	-	60	0.20	200
SMVF-05	40	-	25	-	-	135	0.20	200
SMVF-06	40	-	50	-	-	110	0.20	200
SMVF-07	40	-	75	-	-	85	0.20	200
SMVF-08	40	-	100	-	-	60	0.20	200
SMVF-09	40	-	-	25	-	135	0.20	200
SMVF-10	40	-	-	50	-	110	0.20	200
SMVF-11	40	-	-	75	-	85	0.20	200
SMVF-12	40	-	-	100	-	60	0.20	200
SMVF-13	40	-	-	-	25	135	0.20	200
SMVF-14	40	-	-	-	50	110	0.20	200
SMVF-15	40	-	-		75	85	0.20	200
SMVF-16	40	-	-	-	100	60	0.20	200

PROCESS PARAMETERS OPTIMIZATION

Parameters	***Observation***	***HPMC & EC microspheres***	***Carbopol 940 microspheres***	***PLGA microspheres***
Reaction Duration	5 min	Turbid solution	Turbid solution	Hazy solution
	10 min	Hazy solution	Hazy solution	Hazy solution
	20 min	Slight undissolved particles	Undissolved particles	Slight undissolved particles
	30 min	Clear solution	Clear solution	Clear solution
Reaction temperature of polymer	25 ℃	No solubilization	No solubilization	No solubilization
	30℃	Dissolution occurred	Slight dissolution	No dissolution
	40℃	Not performed	Complete dissolution	Complete dissolution
Reaction temperature of API	25 ℃	Complete dissolution	Complete dissolution	Complete dissolution
Washing solvent	Water	Good yield with low stickiness	Stickiness is observed more.	Buff colored precipitates occurred
	15% methanol	Good yield with low stickiness	Good yield and less stickiness	Less stickiness with good yield
	Methanol: Water mixture	Less yield with high moisture	Moisture level is high, removal of solvent is difficult	Little stickiness and decrease in yield obtained
Washing cycle	Initial	Odor of chloroform /ethyl acetate was present	Odor of chloroform was present	Odor of ethyl acetate (Strong) and chloroform
	Water	Very slight odor observed.		
	Methanol (15%)	Acute odor sensed.		
	Methanol: water mixture	Slightly odor sensed.	No odor sensed.	Very less odor sensed.
Drying duration	60 min	Wet precipitate of product with moisture		
	120 min	Moisture was observed		

Parameters	*Observation*	*HPMC & EC microspheres*	*Carbopol 940 microspheres*	*PLGA microspheres*
	240 min	Slight moisture	Moisture level is lowered	Dried product observed.
	300 min	Completely dried with no residual moisture		
Mixing speed	100 RPM	Mixing not effective		
	200 RPM	Lower mixing efficiency		
	250 RPM	Good flow of mixing and breaking of agglomeration observed		
	300 RPM	Splashing of solution & bubble formation observed		

Process flow diagram

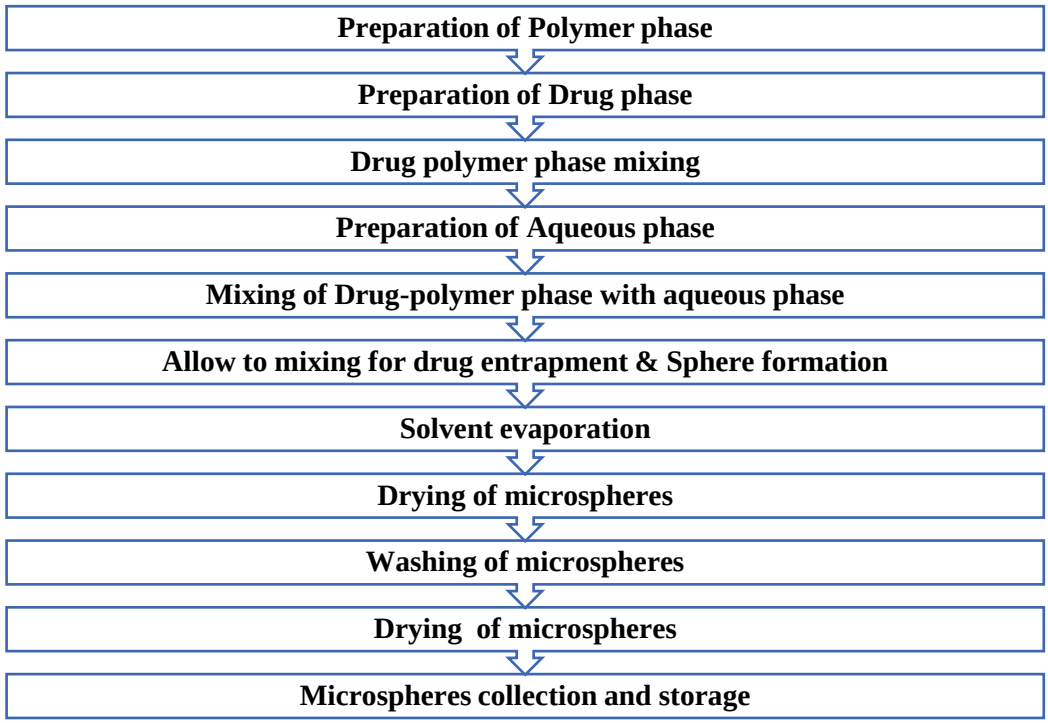


Figure 16: Process flow diagrammatic representation

11.0 CHARACTERIZATION STUDIES

11.1 Organoleptic properties

Colour, odor and texture

About 1.0 gm of sample is taken in a dry Petri dish and the sample is examined for compliance against the specification.

11.2 Yields of microspheres

The prepared microspheres practical yield of various batches was calculated by using the weight of final product after drying with respect to initial total weight of drug and polymer utilized for microsphere formulation. Yield of various batches were calculated by the below mentioned formula

$$\textit{Percentage yield (\%)} = \frac{\textit{Practical yield (Wt. of microspheres obtained)}}{\textit{Theoretical yield (Wt. of Drug+ Polymer taken)}} \textit{ X 100}$$

11.3 Drug content of microspheres

Predetermined quantity of microspheres (40 mg) containing drug was dissolved in 40 mL of methanol and subjected to ultrasonication. The solution was filtered through 0.45 µm PVDF filter and 1.0 mL was transferred to 10 mL volumetric flask. The volume was made up to the mark with methanol. Absorbance was determined by UV spectrophotometer and the drug content was calculated according to the equation.

$$\textit{Percentage drug content (\%)} = \frac{\textit{Weight of drug in microspheres}}{\textit{Weight of microspheres recovered}} \textit{ X 100}$$

11.4 Drug entrapment efficiency

Accurately weighed quantity (40 mg) of drug containing microspheres was dissolved in 40 mL of methanol and shaken for until the dissolution obtained. Upon dissolution 10 mL of phosphate buffer solution (pH 6.8) added and subjected to centrifugation at 2500 RPM for 15 min. After centrifugation simvastatin in the PBS layer was quantified cautiously. Absorbance was determined by UV spectrophotometer and the Entrapment efficiency was calculated according to the equation.

$$\% \textit{ Entrapment efficiency } = \frac{\textit{Calculated drug concentration}}{\textit{Theoretical drug content}} \times 100$$

11.5 Morphology characterization

The shape and surface characteristics of the microspheres were subjected by using scanning electron microscope (SEM).The prepared microspheres were placed directly on the SEM sample holder by using double side fixing tape and coated with gold film under reduced pressure (0.001 torr) and the particles was magnified at specific micrometer and photographed.

11.6 Evaluation of Drug release

***11.6.1 In-vitro* drug release study**

The *in-vitro* drug release study of simvastatin from formulated microspheres carried out in USP type-II apparatus (Paddle type) with the speed of 50 RPM. 900 mL of Phosphate buffer pH 6.8 was used as a dissolution medium. During the *in-vitro* drug release study medium temperature maintained at 37 ± 0.5ºC. At specified interval 03 mL of samples were withdrawn and filtered with the aid of 0.45µ filter. To maintain the sink condition aliquots were replaced with fresh buffer. Samples were assayed by spectrophotometrically at 238 nm.

***11.6.2 In-vitro* drug release study for PLGA microspheres**

The *in-vitro* drug release study of simvastatin from formulated microspheres carried out in USP type-II apparatus (Paddle type) with the speed of 50 RPM. For PLGA microspheres 900 mL of Phosphate buffer pH 6.8 was used as a dissolution medium along with 0.1% SLS concentration for enhance the drug solubility. During the *in-vitro* drug release study release medium temperature maintained at 37 ± 0.5ºC.At specified interval 03 mL of samples were withdrawn and filtered with the aid of 0.45µ filter. To maintain the sink condition aliquots were replaced with fresh buffer. Samples were assayed by spectrophotometrically at 238 nm.

11.7.0 Pharmacokinetic studies of Drug release

To establish the drug release mechanism (release kinetic) from the drug product obtained from the value were fitted with zero order, First order, Higuchi's model and Hixson model. From the obtained data with the kinetic study R^2 values (regression coefficient) was calculated and investigated.

11.7.1 Zero order release rate kinetics

To study the Zero order release kinetics , the drug release rate was fitted to the equation

$$C=K0.t$$

Where,

F : drug release
K0 : Zero order rate constant
t: time for drug release
C: % drug released

A graph was plotted with % drug released vs. Time

11.7.2 First order release kinetics

To study the first order release kinetics , the drug release rate was fitted to the equation

$$\text{Log } C=\log C0-Kt /2.303$$

Where,
C0 - Initial concentration of drug
K:First order constant
t :time.
A graph was plotted with log cumulative % drug remaining vs. time.

11.7.3 Higuchi kinetics

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

If the plot yields a straight line, and the slope is one, then the dosage form is considered to follow Higuchi kinetics of drug release. A graph was plotted with cumulative % drug released vs. square root of time

$$Q=Kt^{½}$$

Where K : differential rate constant t - time.

11.7.4 Hixson Crowell model

Hixson Crowell erosion equation used to evaluate the drug release with changes in the surface area and the diameter of particles, the data were plotted. A graph was plotted with cube root of % drug remaining vs. time.

$$Q0^{1/3} - Qt^{1/3} = KHC \times t$$

Where,

Qt :Amount of drug released

t: time

Q0: Initial amount of drug

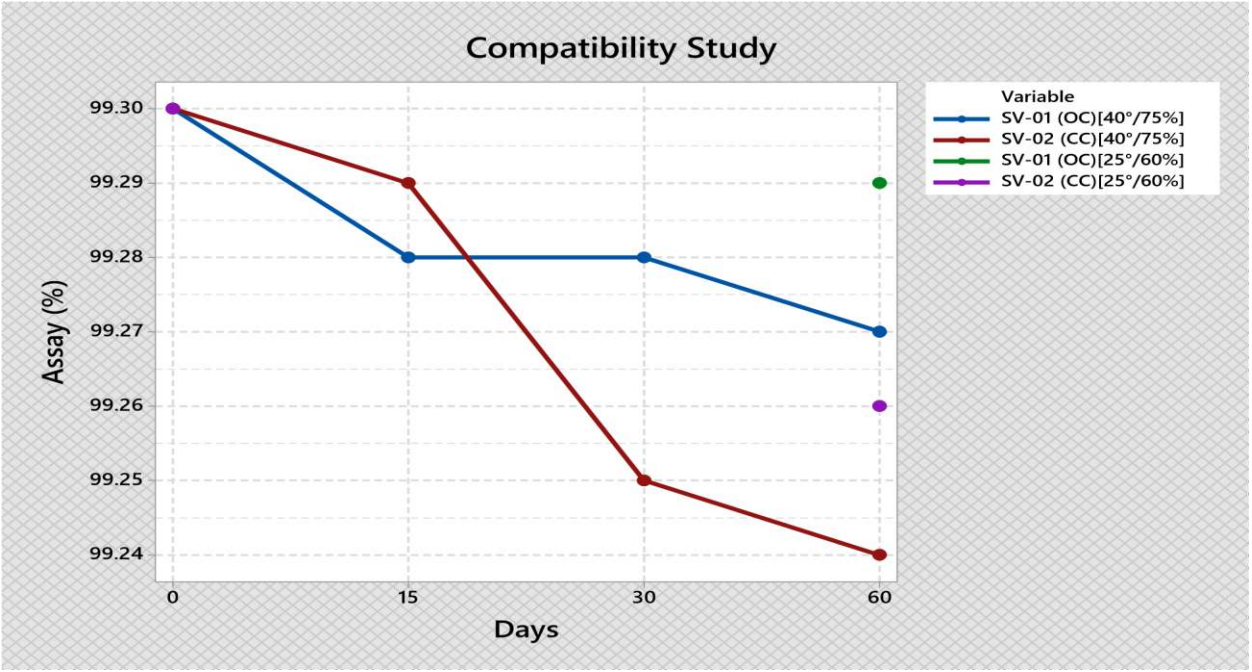
KHC:Rate constant for Hixson Crowell equation.

11.7.5 Stability testing

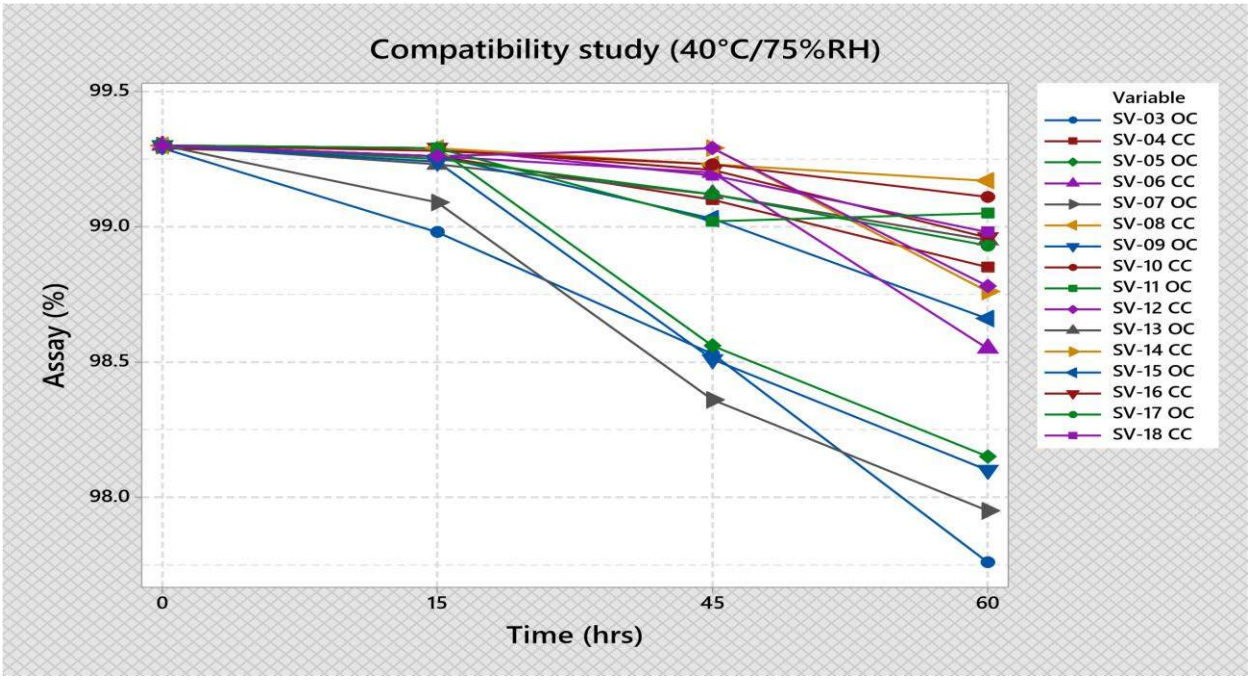
According to IH Q1A (R2),the optimized formulations were stored in stability chamber under the conditions of 40^{o}C/75% RH for a period of 02 months. Then the samples were analyzed spectrophotometrically. The initial prepared samples are used as control.

12.0 CHARACTERIZATION STUDY RESULTS

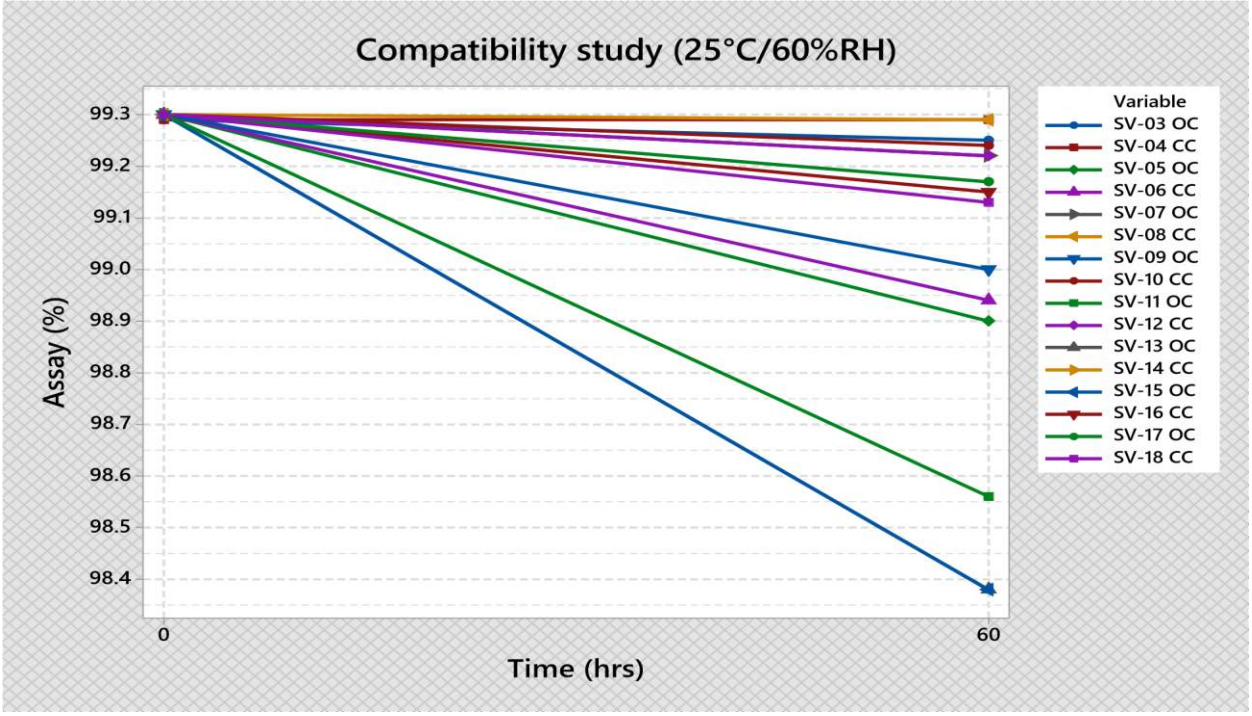
12.1 Drug-Excipient compatibility studies



Graph 01 : Stability study of Simvastatin drug substance



Graph 02 : Stability study of Drug-Excipient mixtures SV-03 to 18 at 40°C/75%RH



Graph 03 : Stability study of Drug-Excipient mixtures SV-03 to 18 at 25°C/60%RH

12.2. Organoleptic property (colour/odor/Texture)

Organoleptic property ↓ Formulation →	SMV loaded HPMC microspheres	SMV loaded Ethyl cellulose microspheres	SMV loaded Carbopol microspheres	SMV loaded PLGA microspheres
Colour	White Puff coloured	Slight white puff colored	White coloured product	Tan coloured product
Odor	Slight pleasant smell	Slight pleasant smell	Slight pleasant smell	Slight pleasant smell
Texture	Free flow powder	Free flow powder	Slight stickiness	Free flow powder

Table 01: Organoleptic properties of prepared microsphere formulation

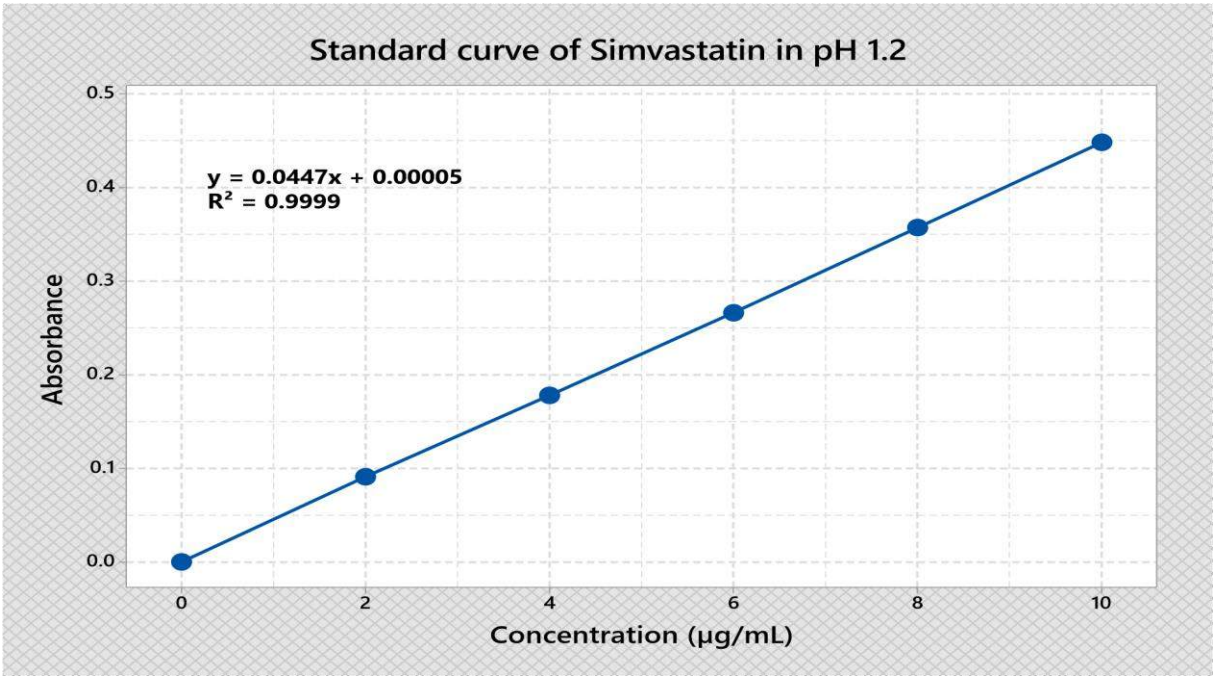
12.3.0 SPECTROSCOPICAL STUDIES

12.3.1.Determination of λmax by UV spectroscopy

12.3.1.1 Calibration of Simvastatin in 0.1 N Hydrochloric acid at 238 nm

Table 02: Calibration curve of Simvastatin in 0.1 N HCL pH 1.2

S.No	Concentration (µg/mL)	Absorbance
1	0	0.0
2	2	0.151
3	4	0.315
4	6	0.463
5	8	0.621
6	10	0.760

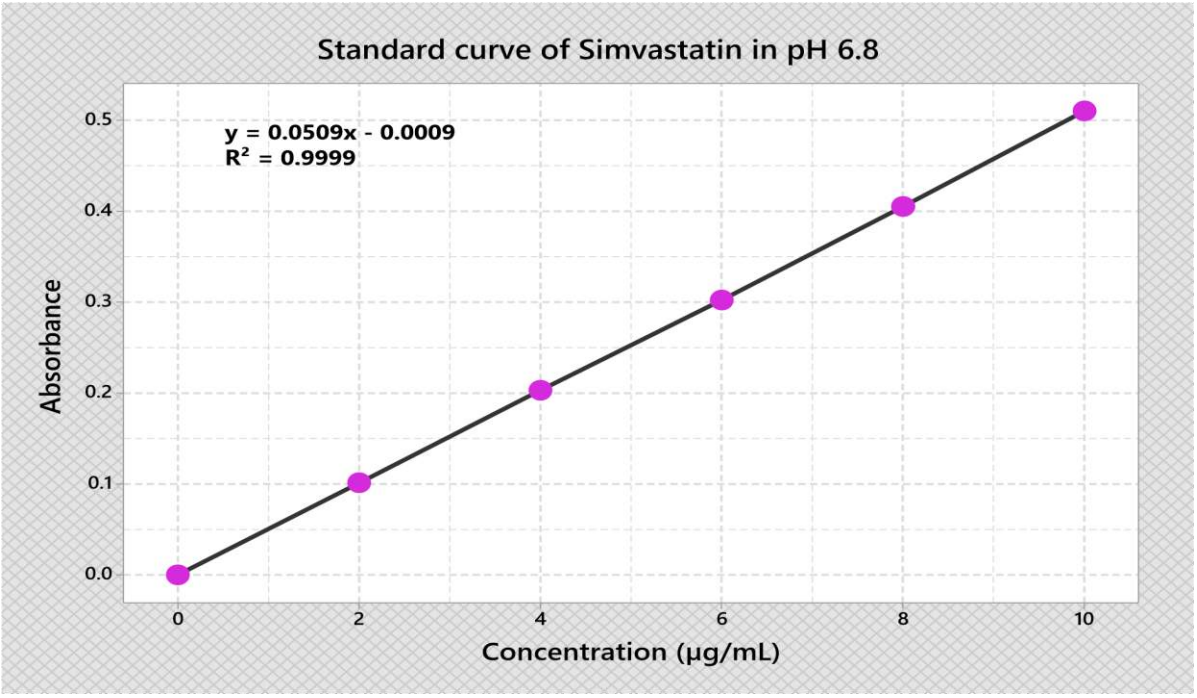


Graph 04: Standard calibration curve of Simvastatin in 0.1 N Hcl

12.3.1.2 Calibration of Simvastatin in pH 6.8 Phosphate buffer at 238 nm

S.No	Concentration (µg/mL)	Absorbance
1	0	0.000
2	2	0.101
3	4	0.203
4	6	0.302
5	8	0.405
6	10	0.510

Table 03: Calibration curve of Simvastatin in 6.8 pH phosphate buffer



Graph 05 : Standard calibration curve of Simvastatin in 6.8 pH phosphate buffer

12.3.2.INFRARED SPECTRUM INTERPRETATION

Table 04 : Characterization of peak in FT-IR spectrum of Pure Simvastatin

Standard wave number (cm^{1})	Observed peaks (cm^{-1})	Functional groups
3550	3550.01	Free OH stretching
3011	3010.82	Olefinic C-H Stretching
2924	2929.71	Methyl C-H symmetric Stretching
2969	2968.32	Methyl C-H asymmetric Stretching
2871	2872.28	Methylene C-H symmetric Stretching
1450	1451.60	C-H bending
1461	1466.52	Methylene C-H symmetric Stretching
1389	1389.97	Gem-dimethyl C-H bending
1267	1268.41	Lactone C-O-C bending
1225	1226.32	Lactone C-O-C bending
1166	1165.47	Ester C-O-C bending
1072	1072.34	Secondary alcohol C-O Stretching
1050	1055.50	Secondary alcohol C-O Stretching
870	869.53	Trisubstituted olefinic C-H wag

Table 05 : Characterization of peak in FT-IR spectrum of HPMC

Standard wave number (cm^{-1})	Observed peaks (cm^{-1})	Functional groups
3550 -3200	3449.3	O-H stretching
1626	1630.8	C=C Stretching
1465	1458.13	C-H bending
1372	1376.92	S=O Stretching
1342	1341.64	S=O Stretching
1410	1410.21	S= O Stretching
1124	1121.47	C-O Stretching
1057	1056.02	C-O-C Stretching

Table 06 : Characterization of peak in FT-IR spectrum of Sodium alginate

Standard wave number (cm^{-1})	Observed peaks (cm^{-1})	Functional groups
3550-3200	3400.22	O-H stretching
2925	2929.14	C-H symmetric Stretching
1638	1632.08	C=C Stretching
1417	1416.05	COO symmetric Stretching
1385	1384.3	C-H bending
1275	1268.09	C-O Stretching
1150	1148.28	C-O Stretching
1030	1026.51	C-O-C Stretching
820	818.51	C-H Stretching

Table 07 : Characterization of peak in FT-IR spectrum of Carbopol 940

Standard wave number (cm^{-1})	Observed peaks (cm^{-1})	Functional groups
3400-3300	3415.96	N-H Stretching
2000	2002.17	C=C=C Stretching
1720	1717.15	C=O Stretching
1450	1451.99	C=H bending
1410	1409.24	S=O Stretching
1170	1170.35	C=O Stretching
1050	1049.94	C-O-C Stretching
800	800.26	C-H Stretching

Table 08 : Characterization of peak in FT-IR spectrum of Ethyl cellulose

Standard wave number (cm^{-1})	Observed peaks (cm^{-1})	Functional groups
3100-3000	2898.97	C-H stretching
3000-2800	2876.46	N-H stretching
1745	1748.46	C=O Stretching
1638	1637.81	C=C Stretching
1450	1444.99	C-H bending
1010	1110.76	S=O Stretching
880	882.13	C-H bending
775	778.49	C-I stretching

Table 09 : Characterization of peak in FT-IR spectrum of Poly(lactic-co-glycolic acid)

Standard wave number (cm^{-1})	Observed peaks (cm^{-1})	Functional groups
3400-3300	3436.49	N-H Stretching
2885-3010	2925.41	C-H Stretching
1627	1631.42	COO Stretching
1397	1384.13	C=O bending
1415	1413.32	S=O Stretching
1350	1353.49	S=O Stretching
1342	1338.6	S=O Stretching
1186-1099	1179.93	C-O Stretching
830	829.17	C-H bending
780	780.6	C-H bending

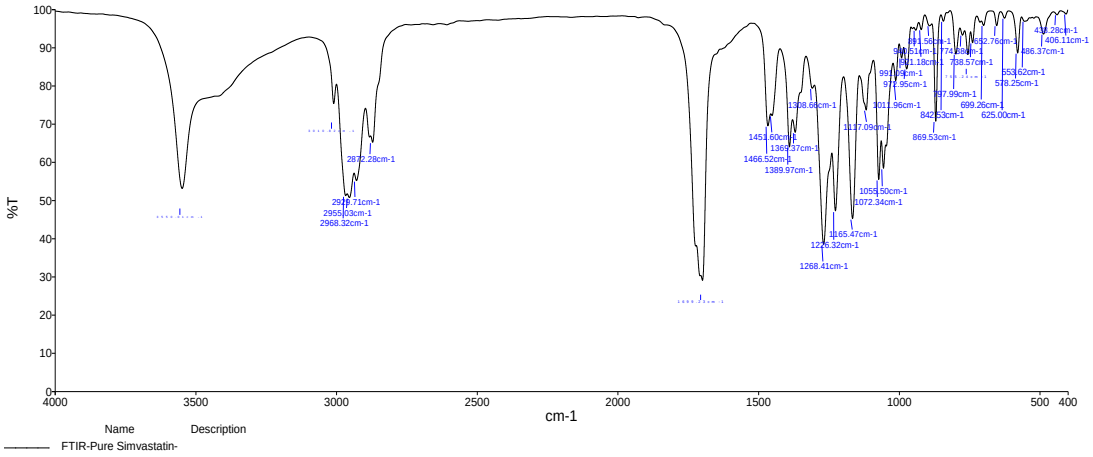


Figure 17 : FT-IR spectrum of Pure Simvastatin

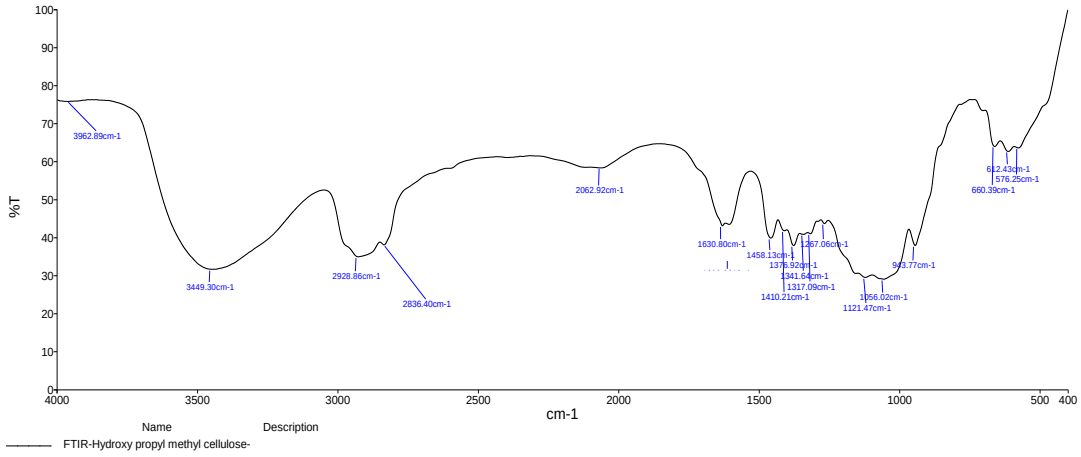


Figure 18 : FT-IR spectrum of HPMC

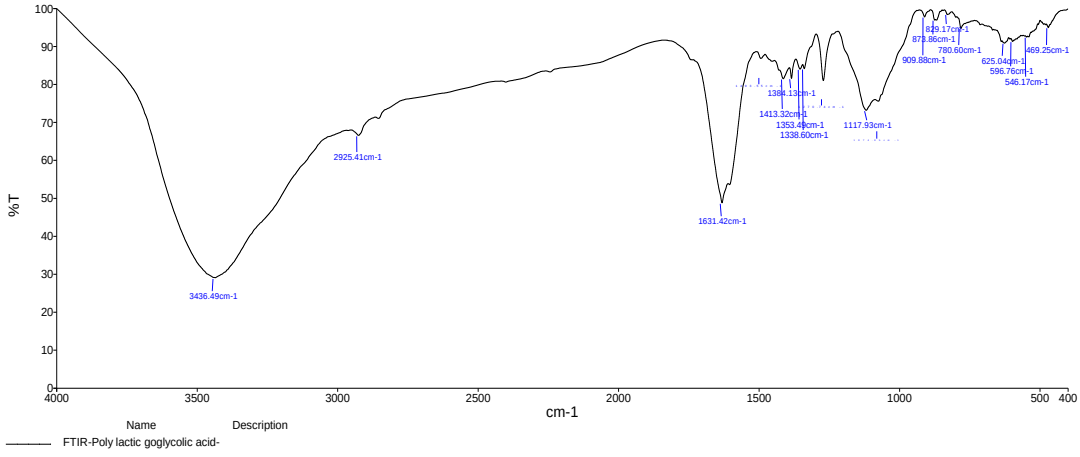


Figure 19 : FT-IR spectrum of Poly lactide co-glycolic acid

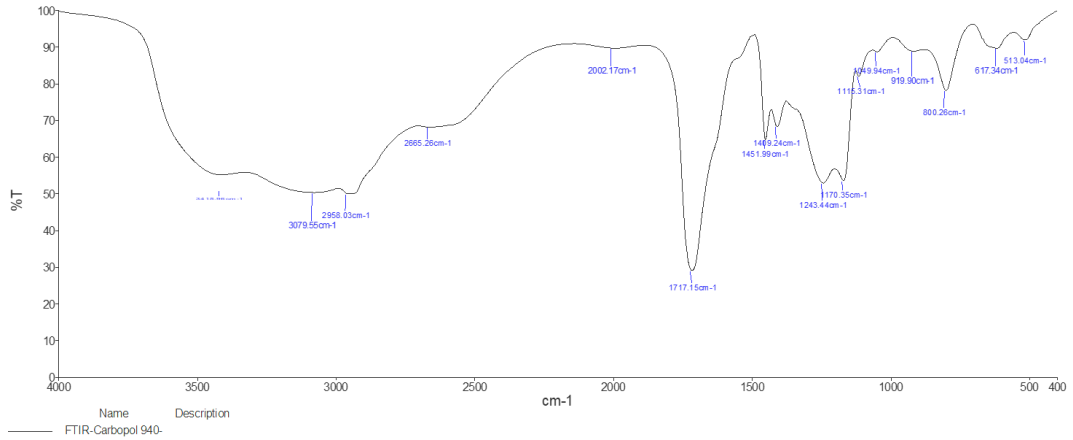


Figure 20 : FT-IR spectrum of Carbopol 940

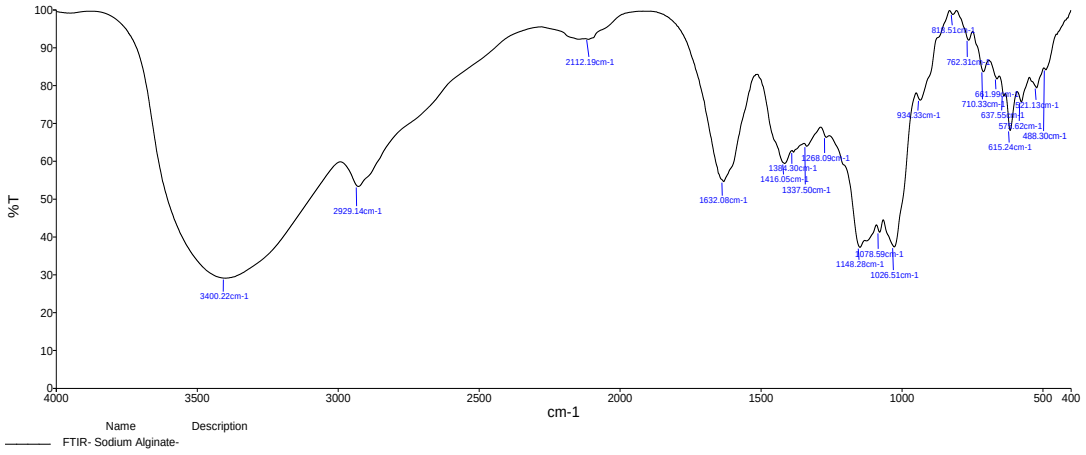


Figure 21 : FT-IR spectrum of Sodium alginate

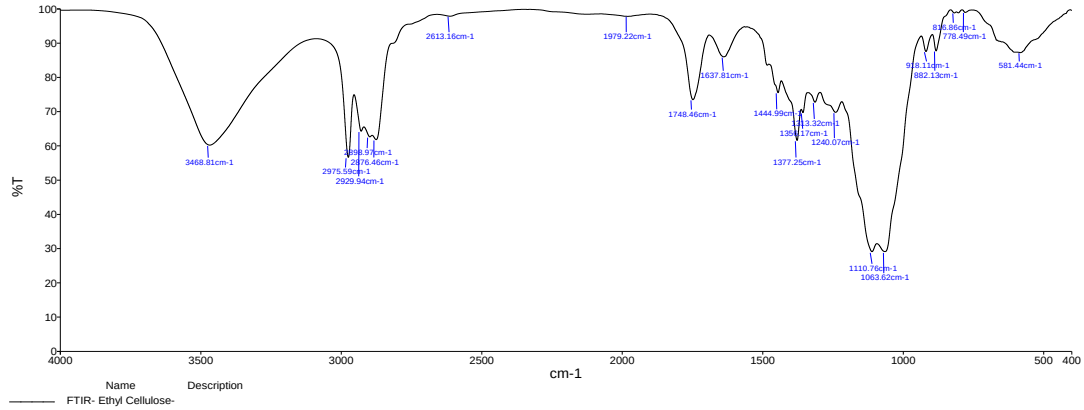


Figure 22 : FT-IR spectrum of Ethyl cellulose

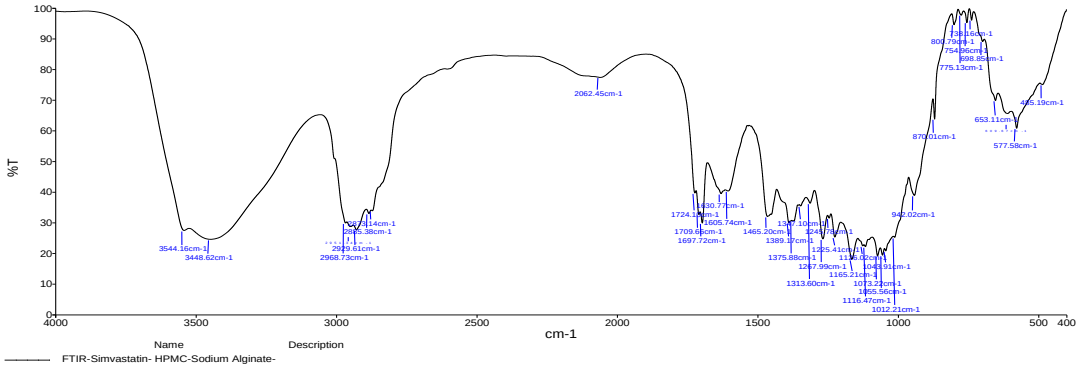


Figure 23 : FT-IR spectrum of Simvastatin + HPMC+ Na-alginate

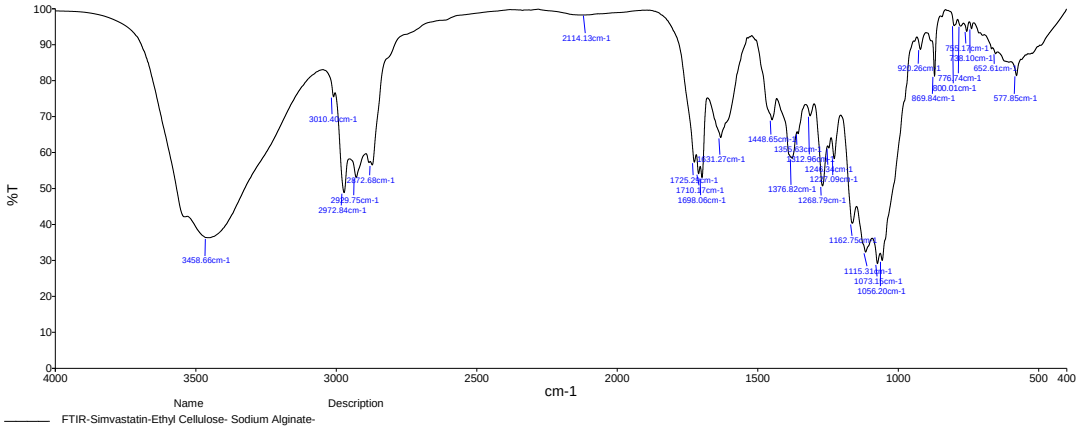


Figure 24 : FT-IR spectrum of Simvastatin + EC+ Na-alginate

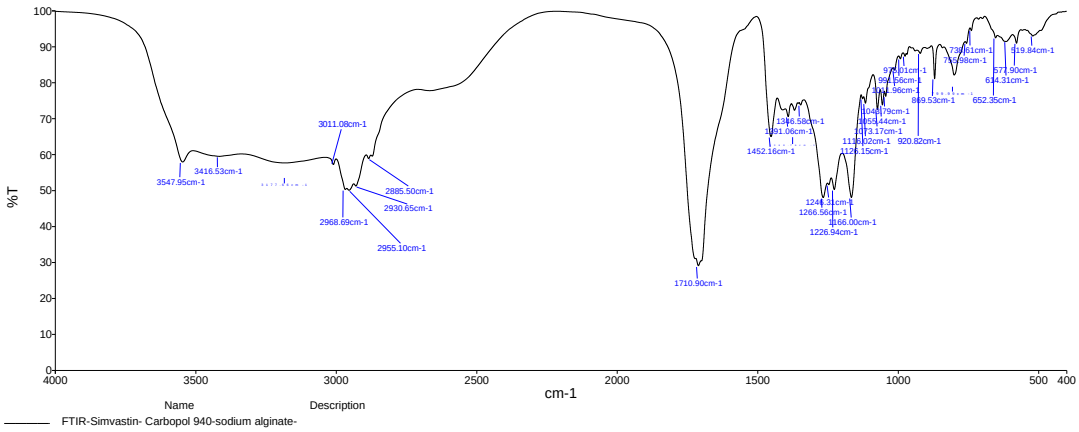


Figure 25 : FT-IR spectrum of Simvastatin + Carbopol 940+ Na-alginate

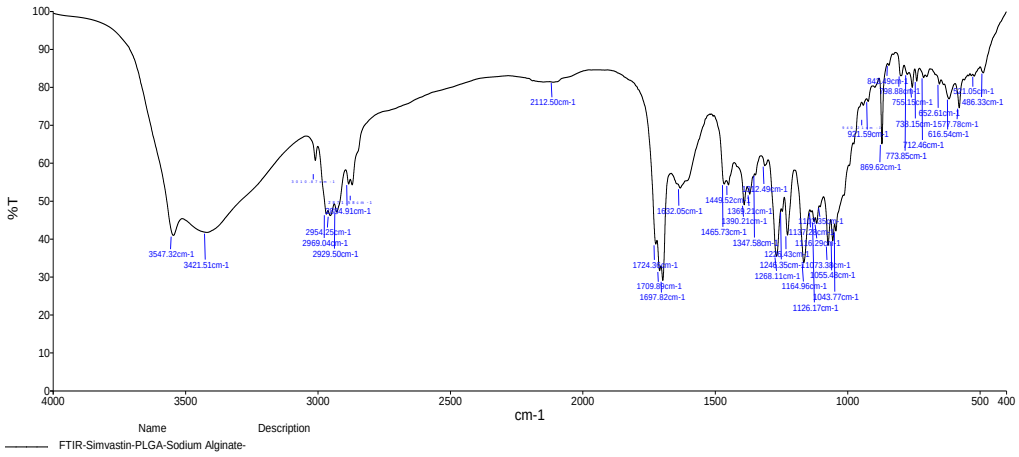


Figure 26 : FT-IR spectrum of Simvastatin + PLGA+ Na-alginate

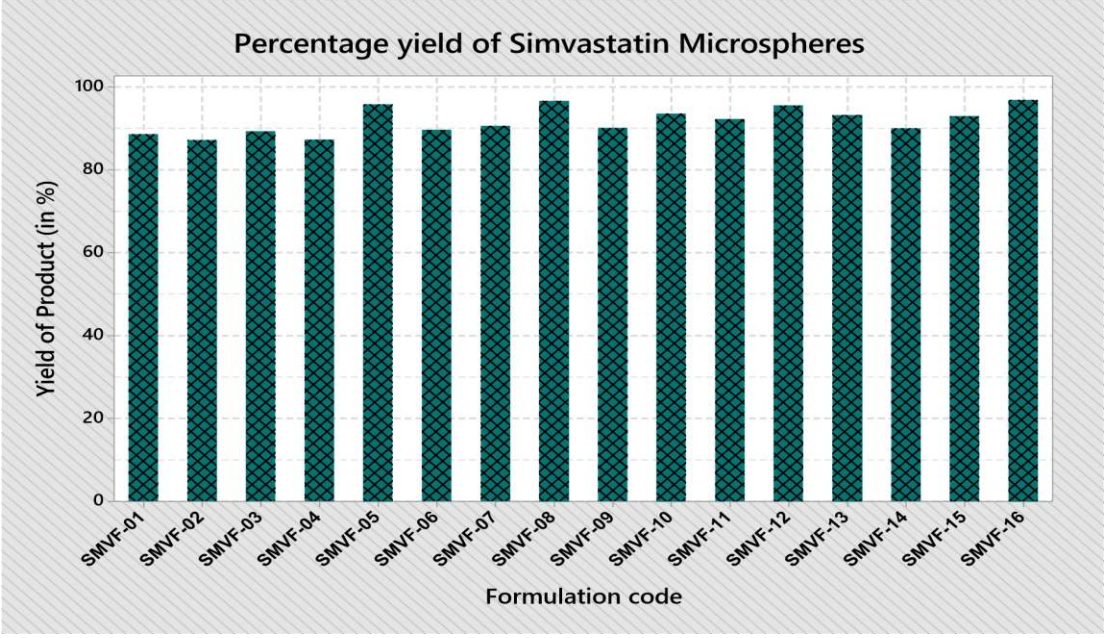
Inference :

To establish the incompatibility study, materials are subjected to Fourier transform infrared spectroscopy of individual material (drug and excipients) and drug excipient mixtures. From the obtained data of spectra analysis resulted in there is no drug-Excipient incompatibles observed with the API and polymer materials. Hence the used materials are compatible and exhibits good consistency.

PERCENTAGE YIELD

Table 10 : Microspheres yield obtained from Formulation SMVF-01 to SMVF-16

S.No	Formulation code	% yield
01	SMVF-01	88.53
02	SMVF-02	87.11
03	SMVF-03	89.20
04	SMVF-04	87.15
05	SMVF-05	95.80
06	SMVF-06	89.57
07	SMVF-07	90.54
08	SMVF-08	96.60
09	SMVF-09	89.99
10	SMVF-10	93.55
11	SMVF-11	92.20
12	SMVF-12	95.53
13	SMVF-13	93.21
14	SMVF-14	89.95
15	SMVF-15	92.93
16	SMVF-16	96.85

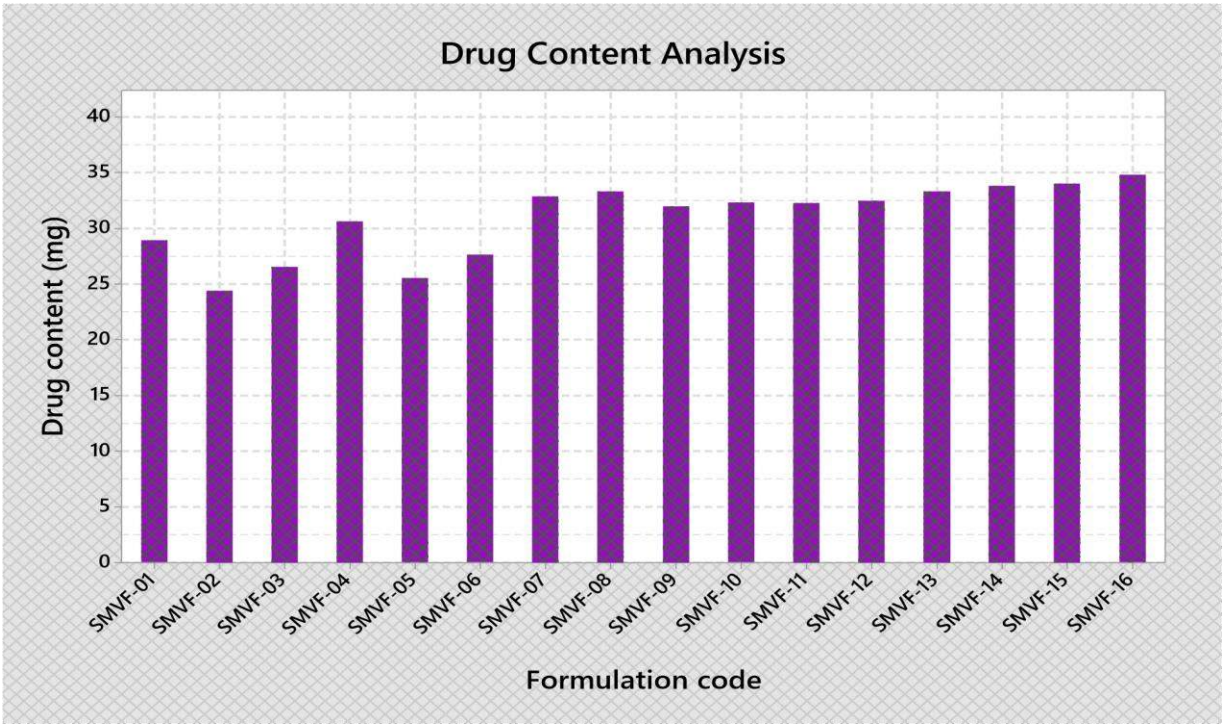


Graph 06 : Percentage yield of microspheres (SMVF-01 to SMVF-16)

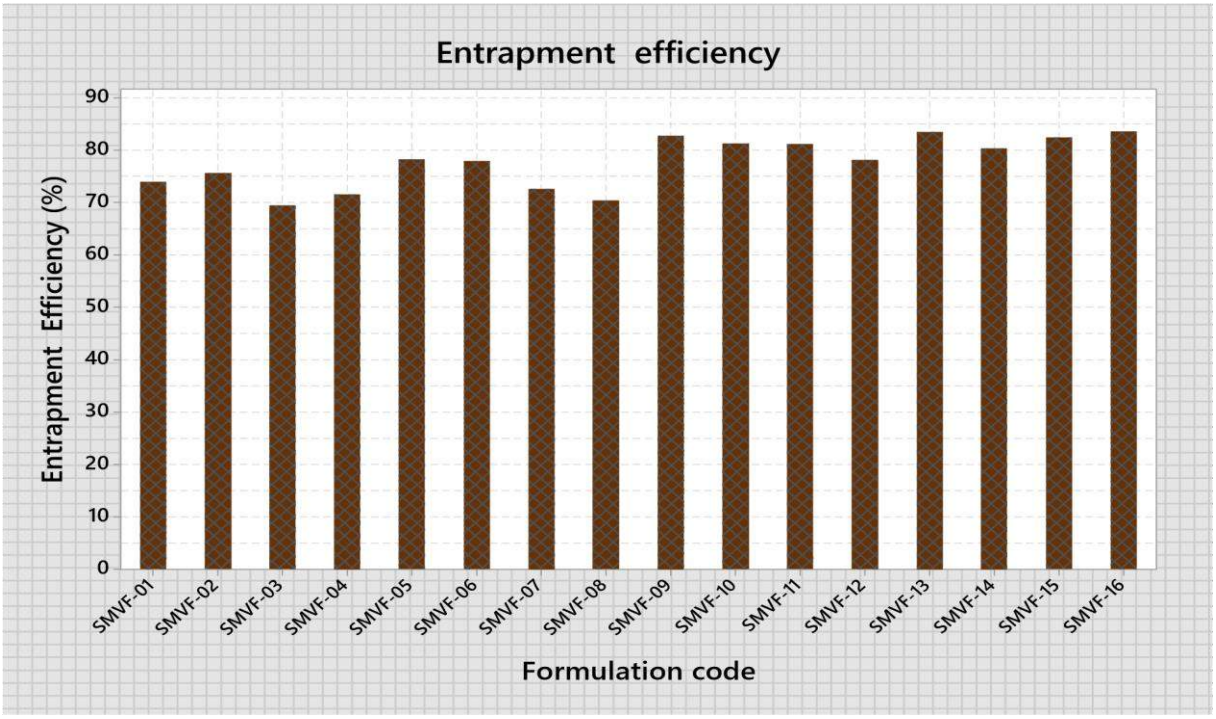
DRUG CONTENT

Table 11 : Drug content loading of Formulation SMVF-01 to SMVF-16

S.No	Formulation code	Drug content	
		Theoretical content (mg)	Actual content (mg)
1	SMVF-01	40.0	28.90
2	SMVF-02	40.0	24.38
3	SMVF-03	40.0	26.53
4	SMVF-04	40.0	30.61
5	SMVF-05	40.0	25.49
6	SMVF-06	40.0	27.60
7	SMVF-07	40.0	32.85
8	SMVF-08	40.0	33.32
9	SMVF-09	40.0	31.95
10	SMVF-10	40.0	32.29
11	SMVF-11	40.0	32.25
12	SMVF-12	40.0	32.47
13	SMVF-13	40.0	33.30
14	SMVF-14	40.0	33.82
15	SMVF-15	40.0	34.01
16	SMVF-16	40.0	34.76



Graph 07 : Drug loading of microspheres (SMVF-01 to SMVF-16)



Graph 08 : Drug entrapment efficiency of microspheres (SMVF-01 to SMVF-16)

DRUG ENTRAPMENT EFFICIENCY

Table 12 :Drug entrapment efficiency of Formulation SMVF-01 to SMVF-16

S.No	Formulation code	Entrapment efficiency (in %)
1	SMVF-01	73.9
2	SMVF-02	75.61
3	SMVF-03	69.38
4	SMVF-04	71.53
5	SMVF-05	78.22
6	SMVF-06	77.85
7	SMVF-07	72.6
8	SMVF-08	70.3
9	SMVF-09	82.77
10	SMVF-10	81.23
11	SMVF-11	81.09
12	SMVF-12	78.1
13	SMVF-13	83.47
14	SMVF-14	80.34
15	SMVF-15	82.4
16	SMVF-16	83.55

MORPHOLOGICAL CHARACTERIZATION

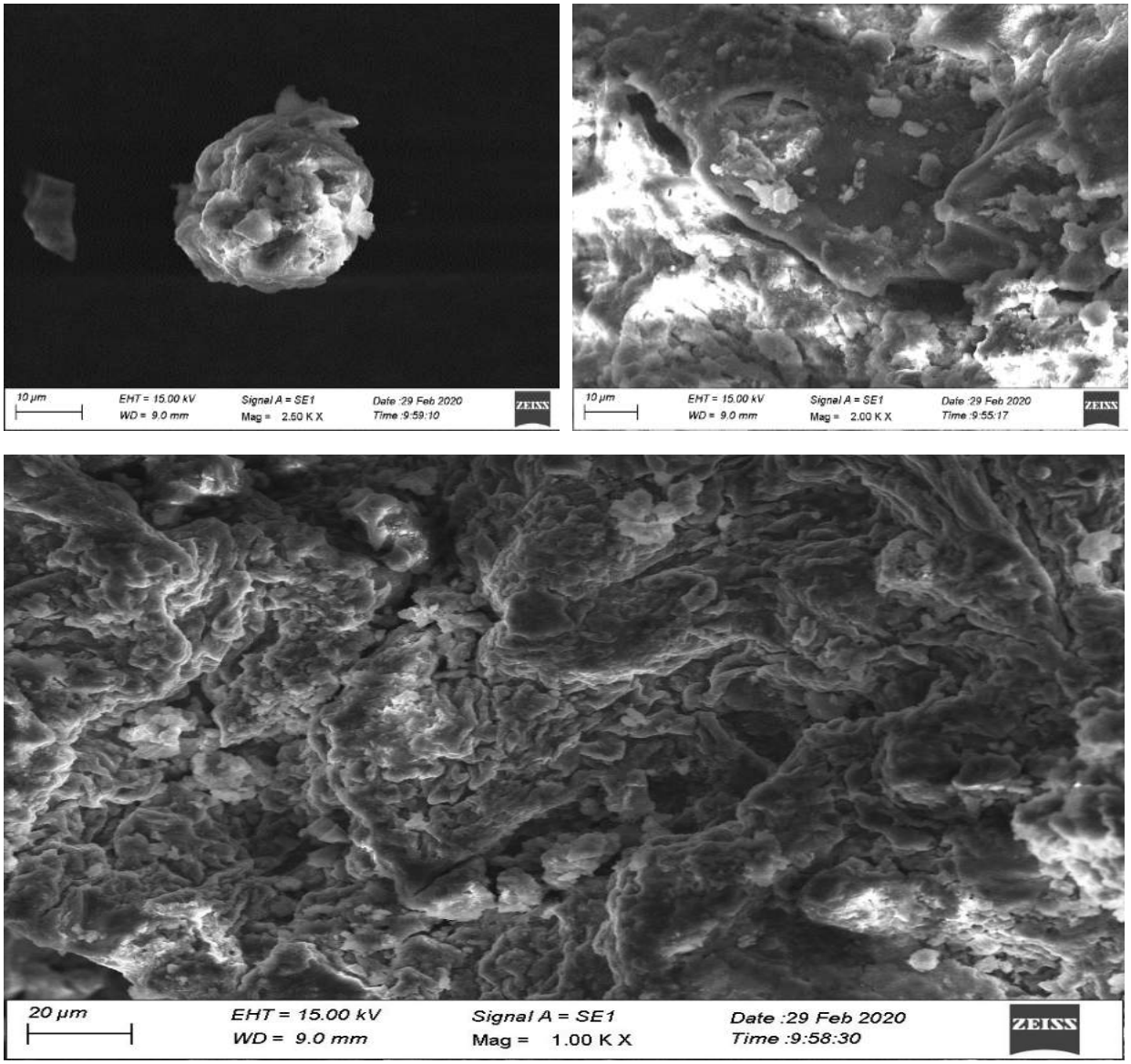


Figure 27 : SEM image of SMV Loaded HPMC Microsphere formulation SMVF-04

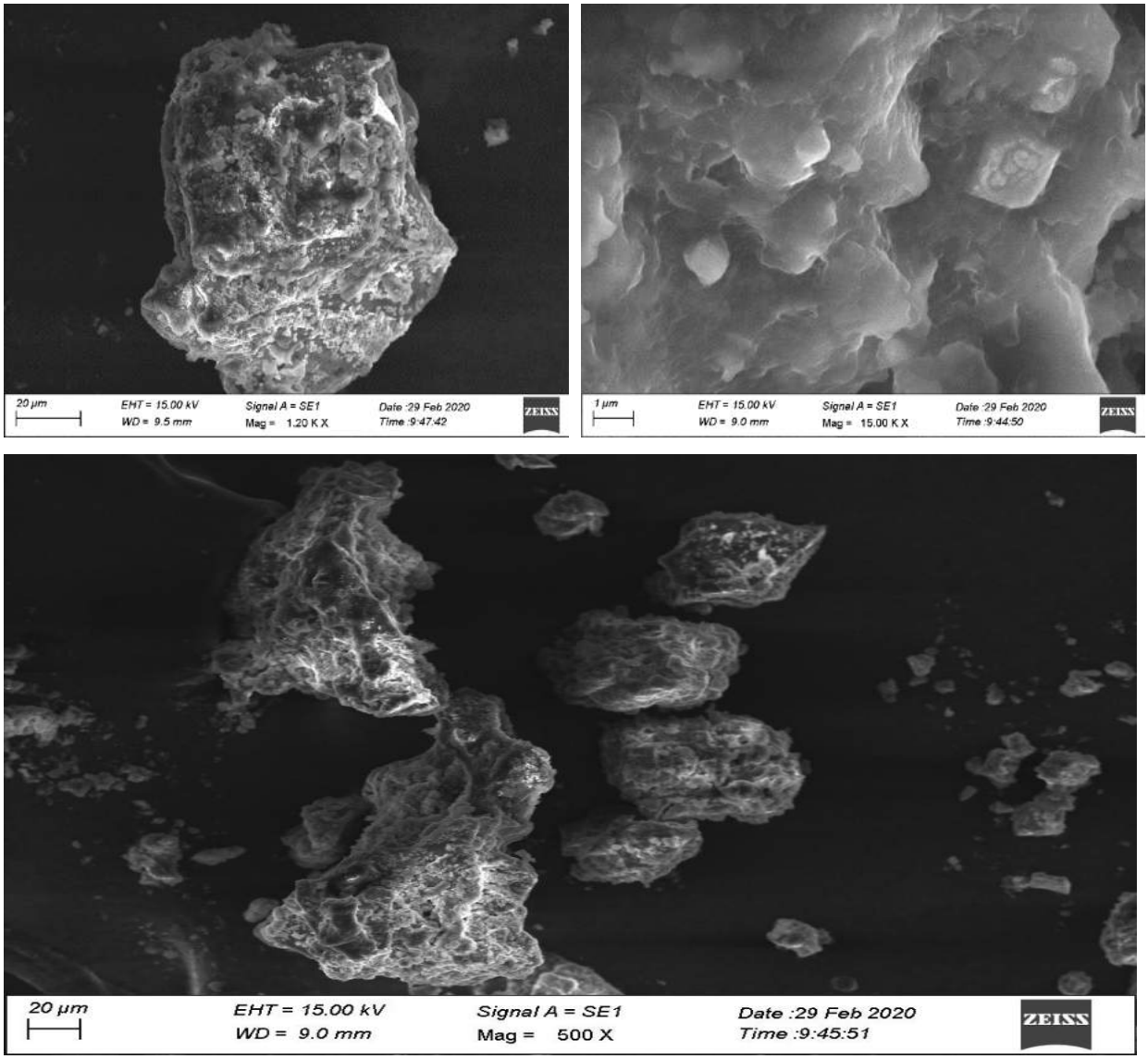


Figure 28 : SEM image of SMV Loaded EC Microsphere formulation SMVF-08

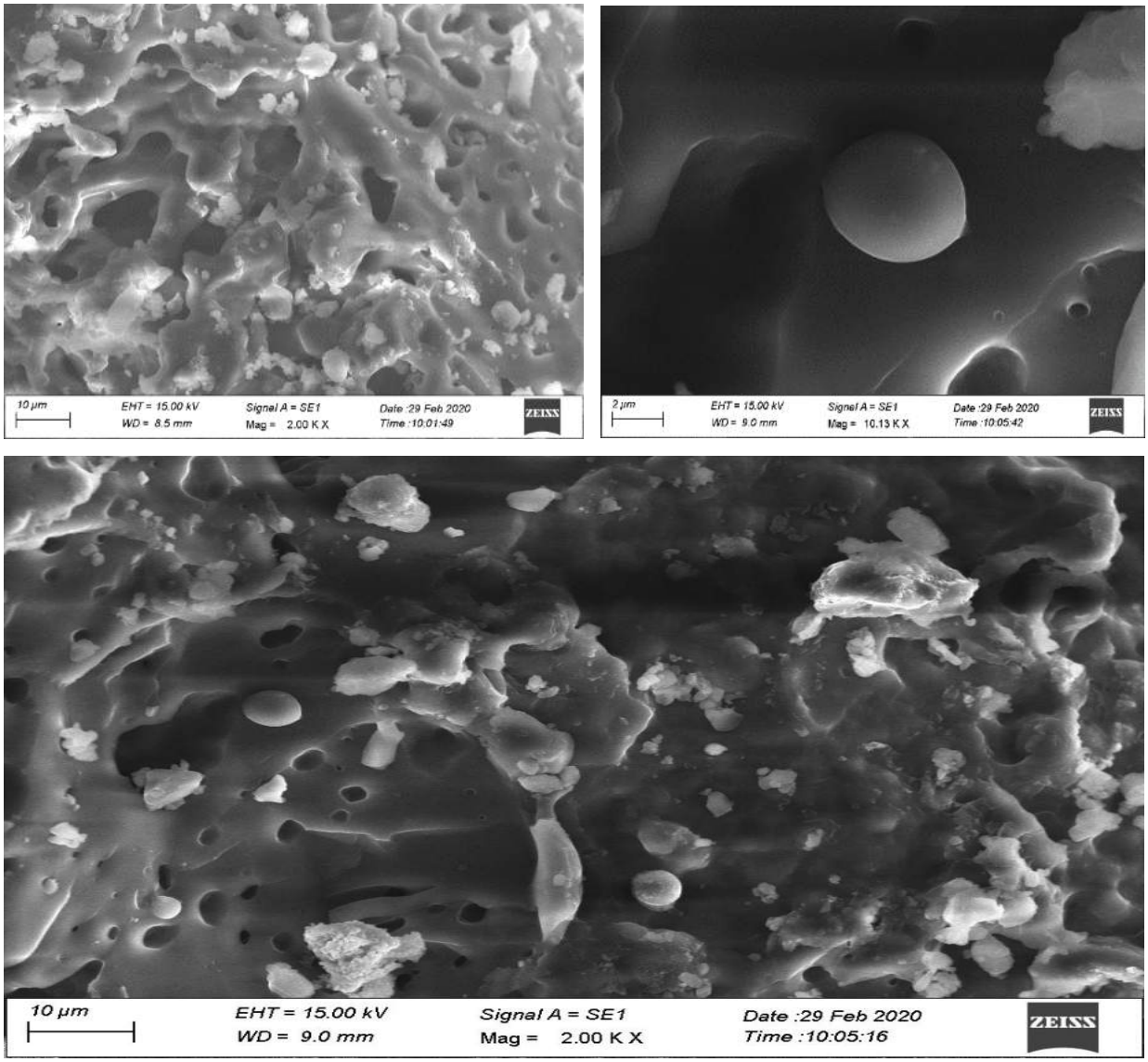


Figure 29 : SEM image of SMV Loaded Carbopol-940 Microsphere formulation SMVF-12

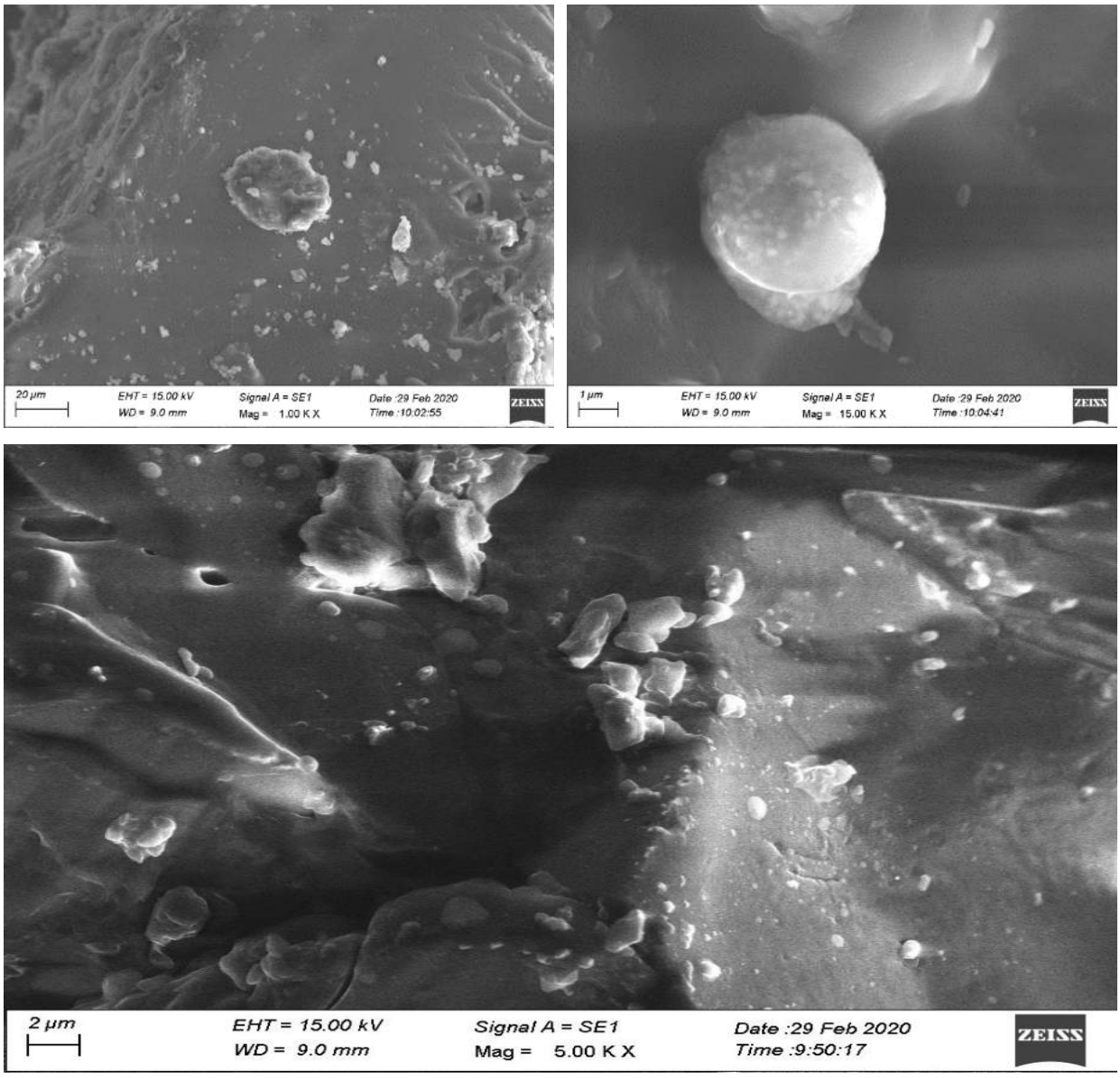
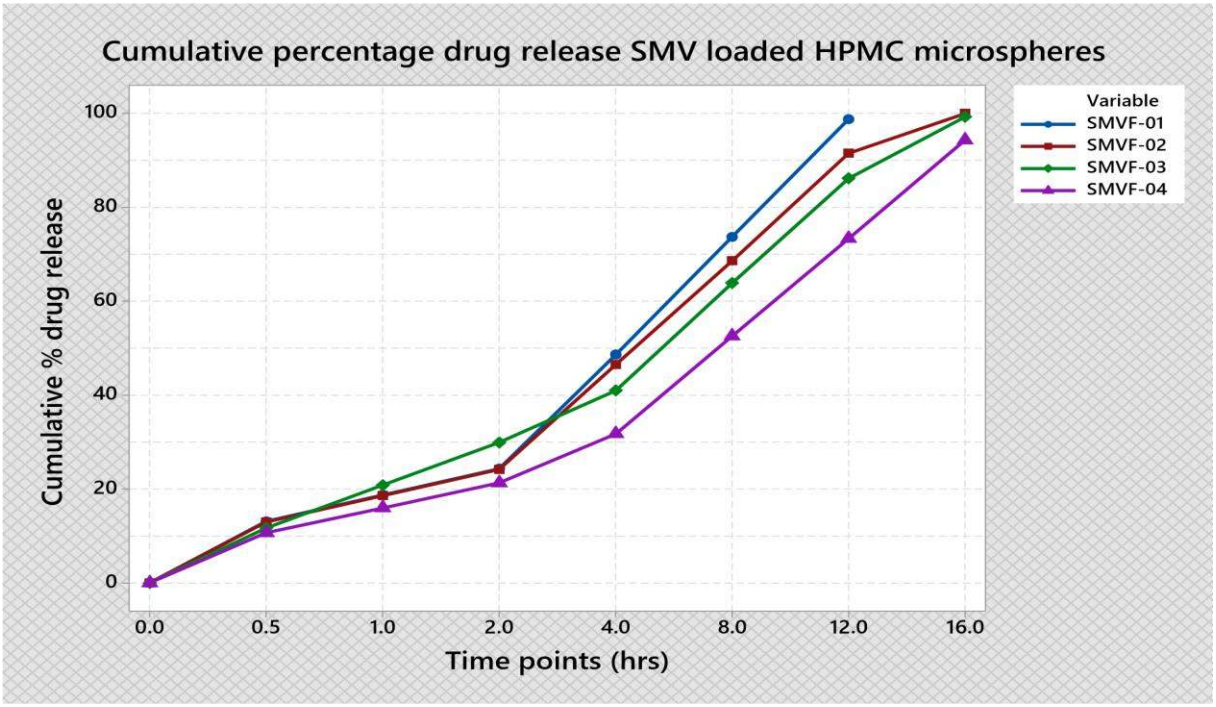


Figure 30 : SEM image of SMV Loaded PLGA Microsphere formulation SMVF-16

IN-VITRO DRUG RELEASE

Table 13 : Cumulative % drug release of simvastatin loaded HPMC microspheres in 6.8 pH phosphate buffer.

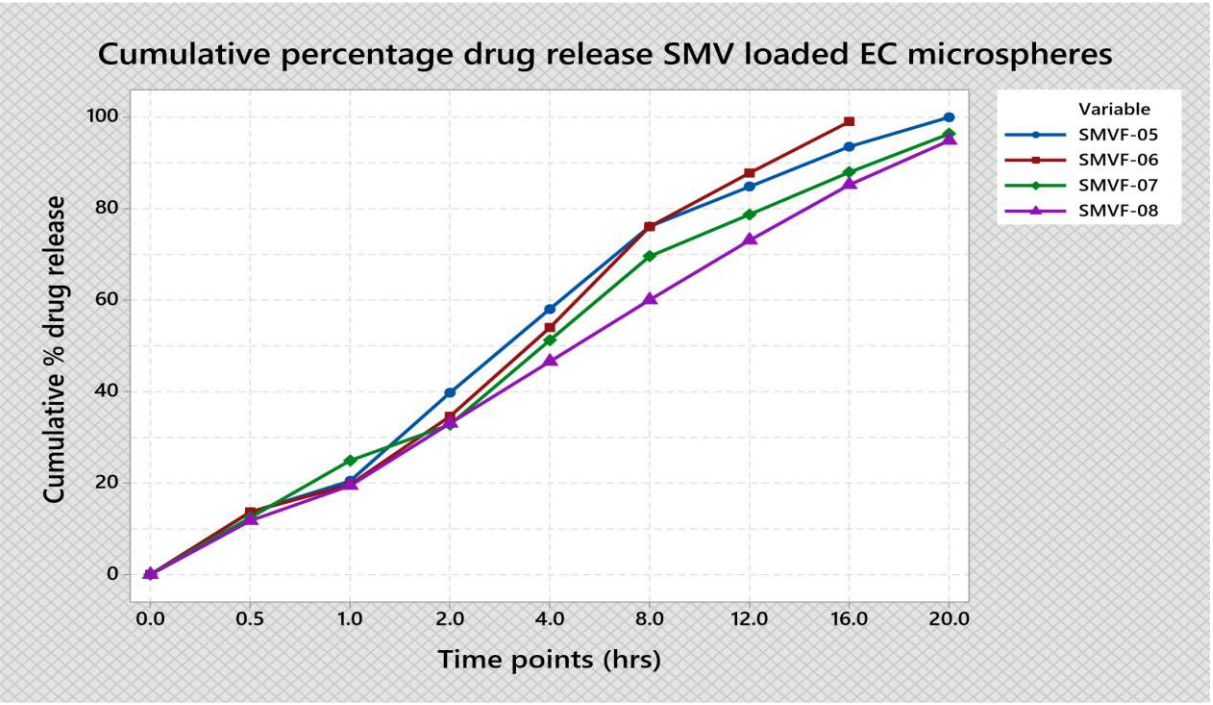
Time points (in hrs)	SMVF-01	SMVF-02	SMVF-03	SMVF-04
0	0	0	0	0
0.5	13.12	12.99	11.71	10.69
1	18.72	18.57	20.80	15.99
2	24.32	24.15	29.89	21.28
4	48.63	46.52	41.00	31.74
8	73.70	68.60	63.85	52.55
12	98.76	91.49	86.12	73.34
16	-	99.98	99.27	94.29



Graph 09 : Cumulative % drug release of formulation SMVF-01 to SMVF--04

Table 14 : Cumulative % drug release of simvastatin loaded Ethyl cellulose microspheres in 6.8 pH phosphate buffer.

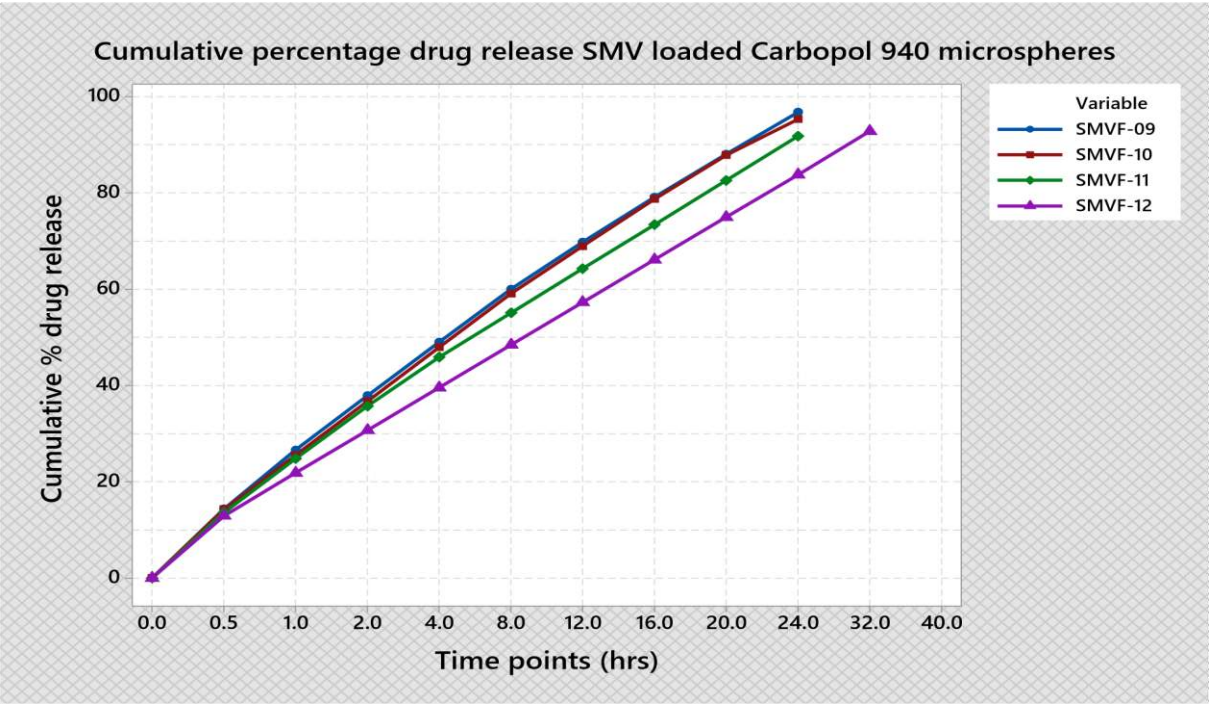
Time points (in hrs)	SMVF-05	SMVF-06	SMVF-07	SMVF-08
0	0	0	0	0
0.5	13.65	13.7	12.5	11.8
1	20.47	19.65	24.91	19.42
2	39.74	34.59	32.75	33.06
4	58.03	53.98	51.22	46.58
8	76.13	76.09	69.62	60.05
12	84.81	87.8	78.74	73.1
16	93.56	99.03	87.96	85.22
20	99.99	-	96.39	94.90



Graph 10 : Cumulative % drug release of formulation SMVF-05 to SMVF-08

Table 15 : Cumulative % drug release of simvastatin loaded Carbopol 940 microspheres in 6.8 pH phosphate buffer.

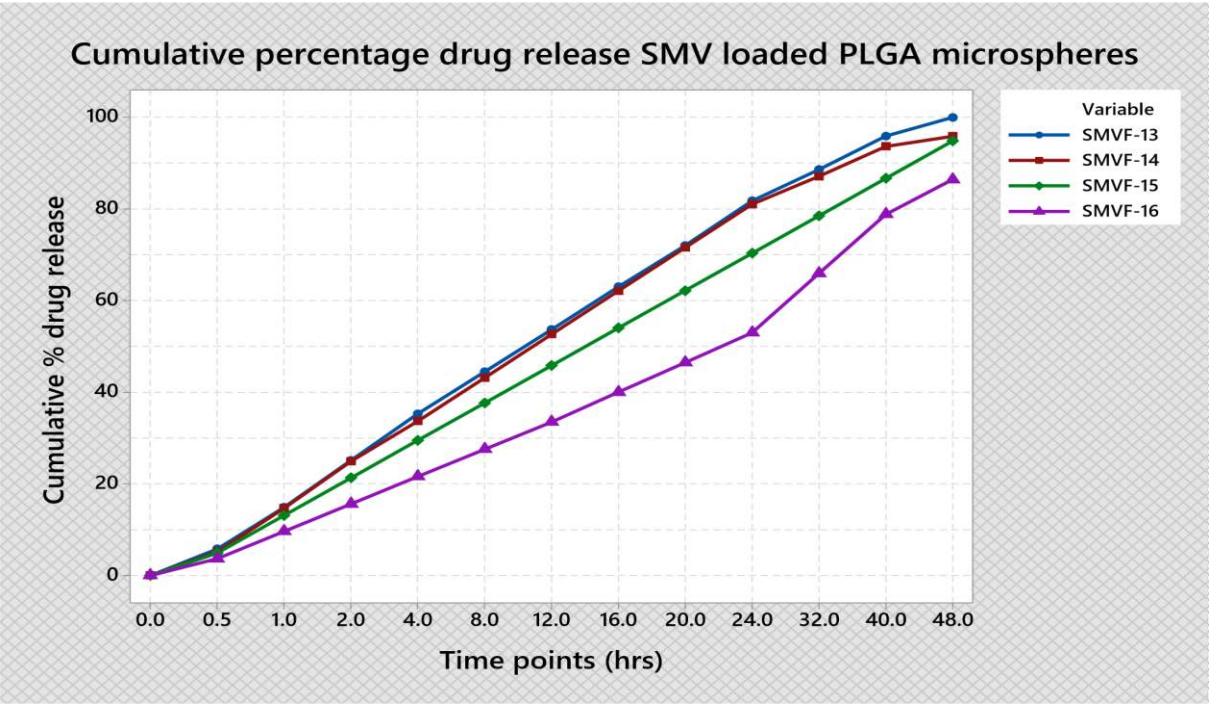
Time points (in hrs)	SMVF-09	SMVF-10	SMVF-11	SMVF-12
0	0	0	0	0
0.5	14.40	14.30	13.65	12.95
1	26.60	25.51	24.80	21.82
2	37.89	36.71	35.71	30.69
4	48.98	47.90	45.89	39.56
8	60.00	59.08	55.08	48.42
12	69.80	68.95	64.26	57.28
16	79.10	78.71	73.42	66.13
20	88.04	87.83	82.57	74.98
24	96.78	95.33	91.79	83.77
32	-	-	-	92.80



Graph 11 : Cumulative % drug release of formulation SMVF-09 to SMVF-12

Table 16 :Cumulative % drug release of simvastatin loaded PLGA microspheres in 6.8 pH phosphate buffer.

Time points	SMVF-13	SMVF-14	SMVF-15	SMVF-16
0	0	0	0	0
0.5	5.8	5.2	4.9	3.65
1	14.9	14.7	13.1	9.63
2	25.1	24.9	21.29	15.62
4	35.29	33.68	29.48	21.59
8	44.48	43.16	37.65	27.55
12	53.67	52.64	45.83	33.49
16	63.20	62.11	54.03	40.00
20	72.02	71.57	62.16	46.49
24	81.75	81.03	70.34	52.97
32	88.6	87.09	78.50	65.92
40	95.89	93.67	86.67	78.86
48	99.97	95.88	94.83	86.41



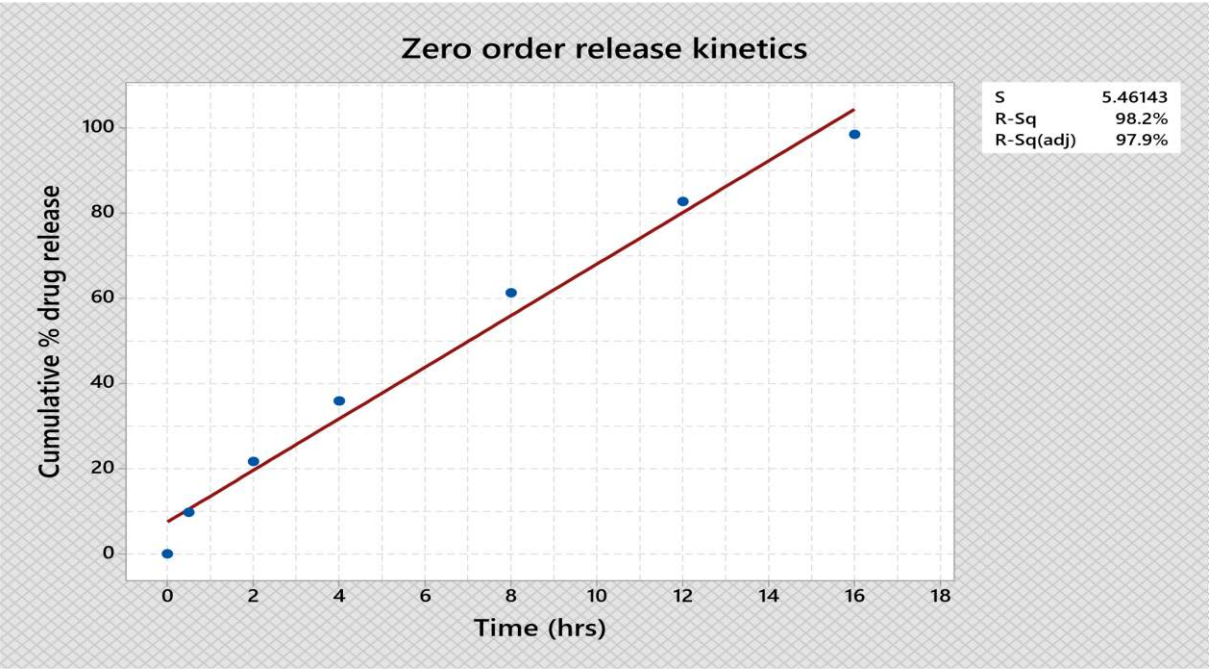
Graph 12:Cumulative % drug release of formulation SMVF-13 to SMVF-16

RELEASE KINETIC (PHARMACOKINETIC STUDY)

Table 17 :Determination of drug release mechanism of optimized formulation SMVF-04

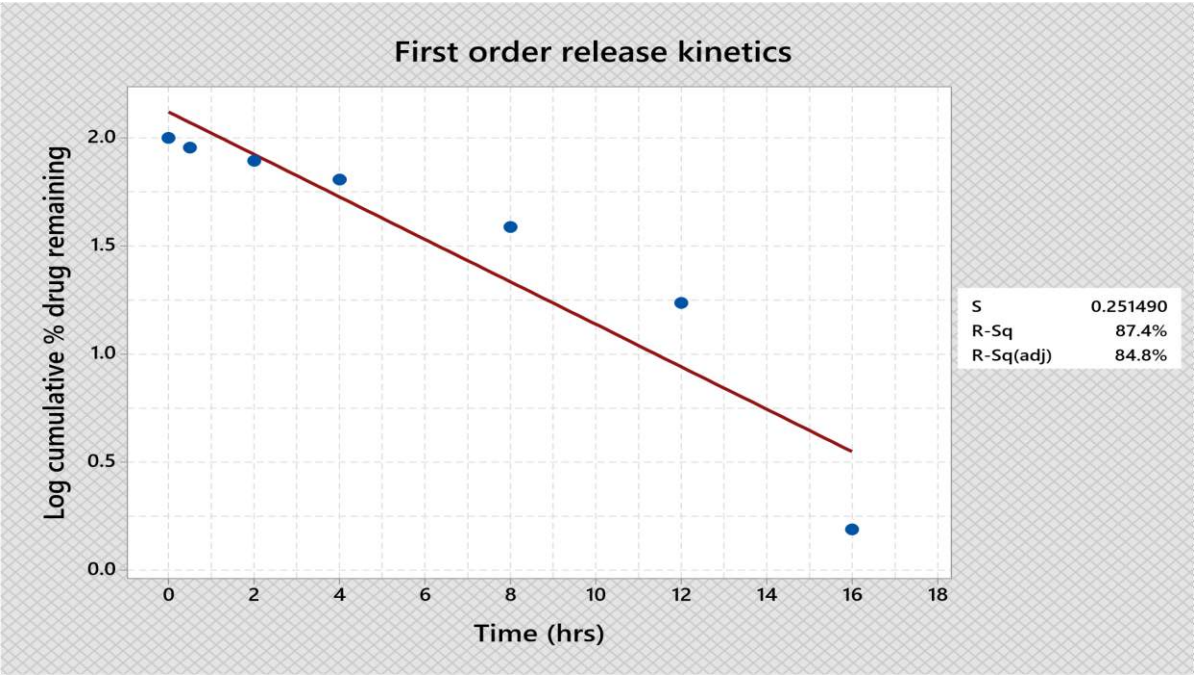
Time (in hrs)	Cumulative percentage drug release	Percentage drug remain	Square root time	Log Cumulative percentage drug remaining	Log time	Log Cumulative percentage drug released	Percentage drug released	Cube Root of % drug Remaining (Wt.)	Wo-Wt.
0	0	100	0.000	2.000	0.000	0.000	0	4.642	0.000
0.5	10.69	89.31	0.707	1.951	-0.301	1.029	10.69	4.470	0.172
1	15.99	84.01	1.000	1.924	0.000	1.204	5.30	4.380	0.262
2	21.28	78.72	1.414	1.896	0.301	1.328	5.29	4.286	0.356
4	31.74	68.26	2.000	1.834	0.602	1.502	10.46	4.087	0.555
8	52.55	47.45	2.828	1.676	0.903	1.721	20.81	3.620	1.022
12	73.34	26.66	3.464	1.426	1.079	1.865	20.79	2.987	1.654
16	94.29	5.71	4.000	0.757	1.204	1.974	20.95	1.787	2.854

Zero order



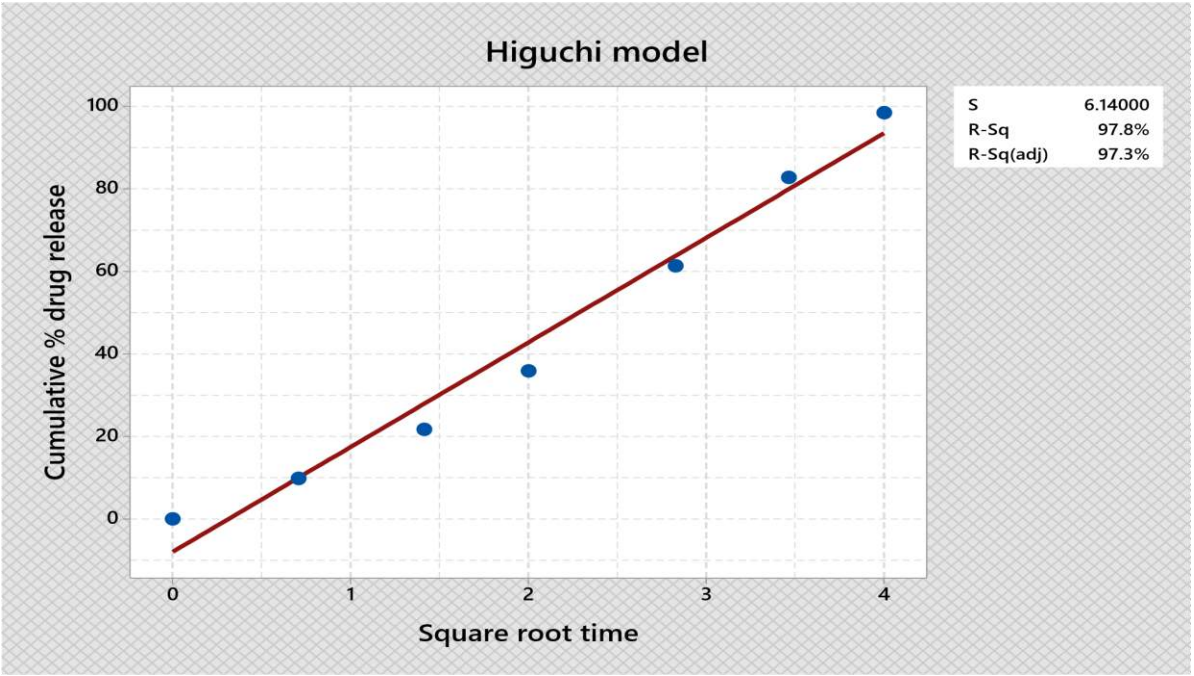
Graph 13 : Zero order drug release of optimized formulation SMVF-04

First order



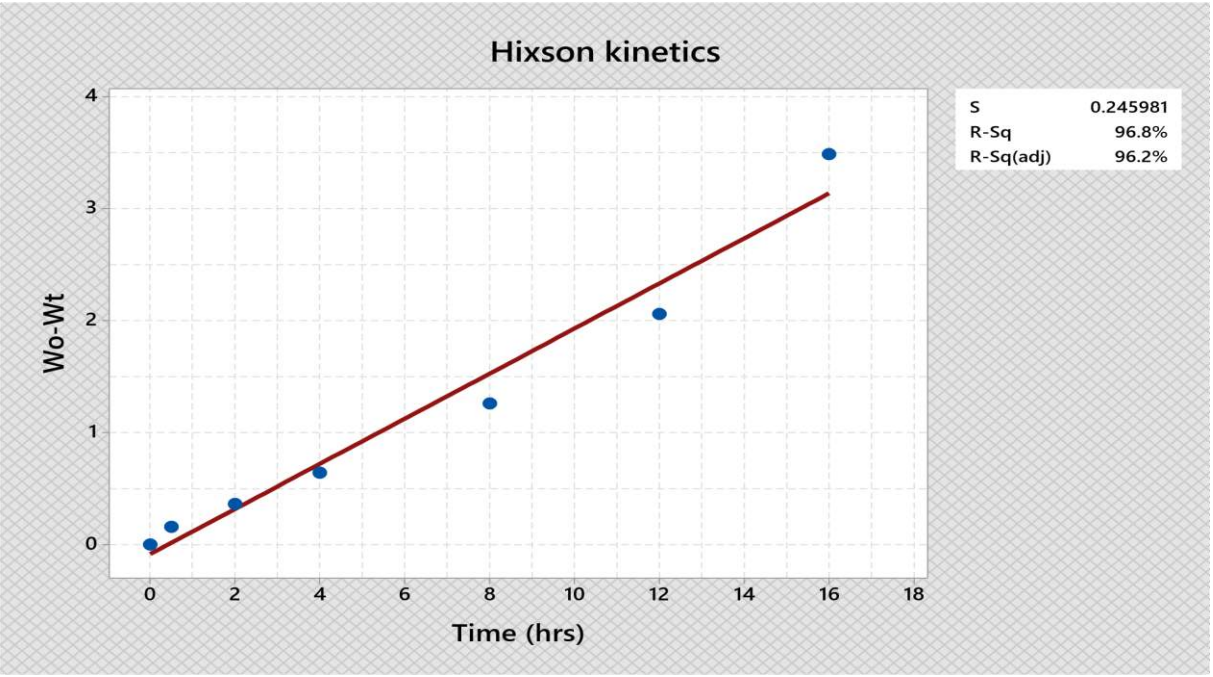
Graph 14 : First order drug release of optimized formulation SMVF-04

Higuchi model



Graph 15 : Higuchi model kinetics for optimized formulation SMVF-04

Hixson model

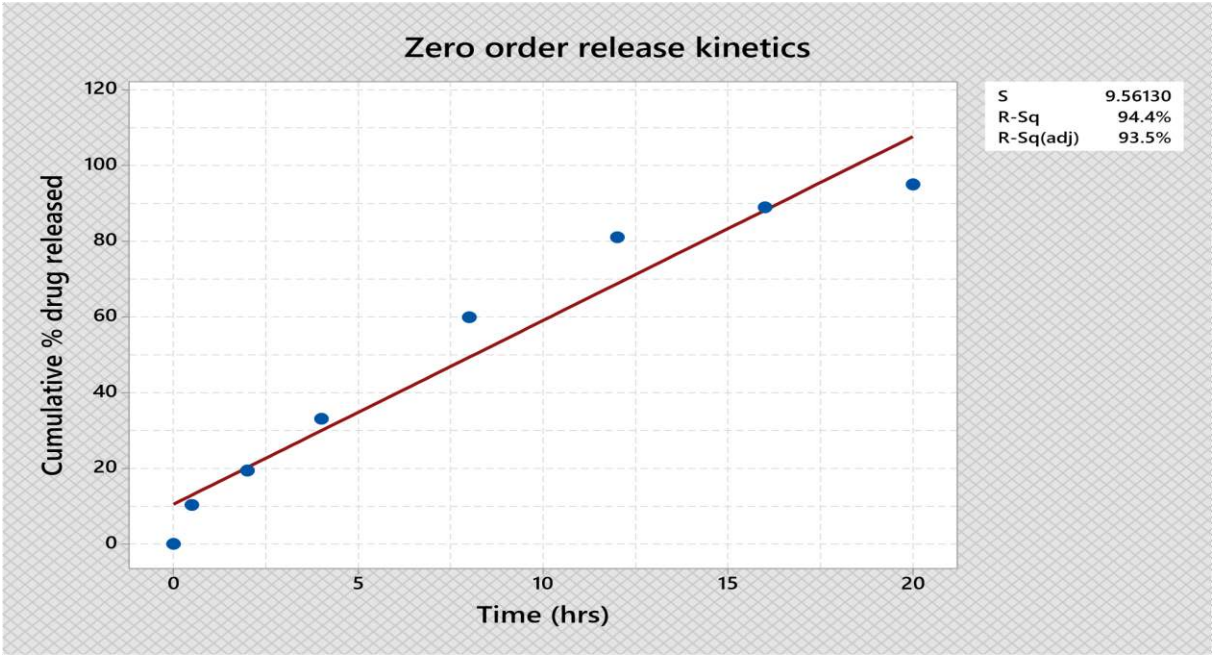


Graph 16 : Hixson model kinetics for optimized formulation SMVF-04

Table 18 :Determination of drug release mechanism of optimized formulation SMVF-08

Time (in hrs)	Cumulative percentage drug release	Percentage drug remain	Square root time	Log Cumulative percentage drug remaining	Log time	Log Cumulative percentage drug released	Percentage drug released	Cube Root of % drug Remaining (Wt.)	Wo-Wt.
0	0	100	0.000	2.000	0.000	0.000	0	4.642	0.000
0.5	10.33	89.67	0.707	1.953	-0.301	1.014	10.33	4.476	0.166
2	19.42	80.58	1.414	1.906	0.301	1.288	9.09	4.319	0.323
4	33.1	66.9	2.000	1.825	0.602	1.520	13.68	4.060	0.582
8	59.87	40.13	2.828	1.603	0.903	1.777	26.77	3.424	1.218
12	81.05	18.95	3.464	1.278	1.079	1.909	21.18	2.666	1.976
16	88.96	11.04	4.000	1.043	1.204	1.949	7.91	2.227	2.414
20	95.02	4.98	4.472	0.697	1.301	1.978	6.06	1.708	2.933

Zero order

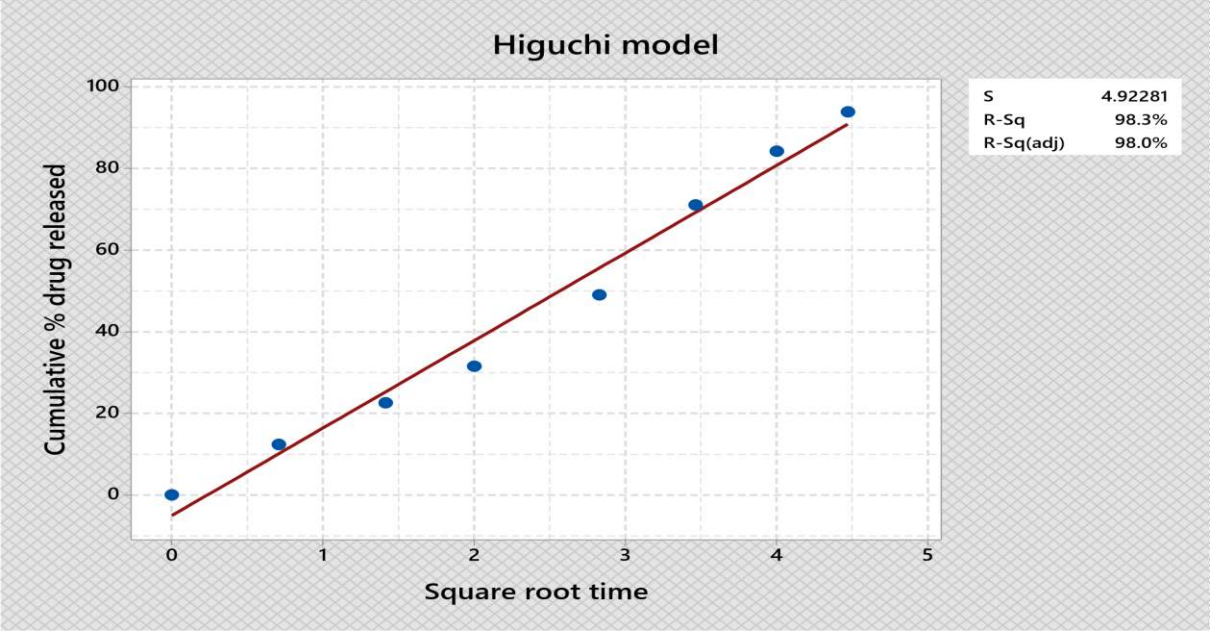


Graph 17 : Zero order drug release of optimized formulation SMVF-08

First order

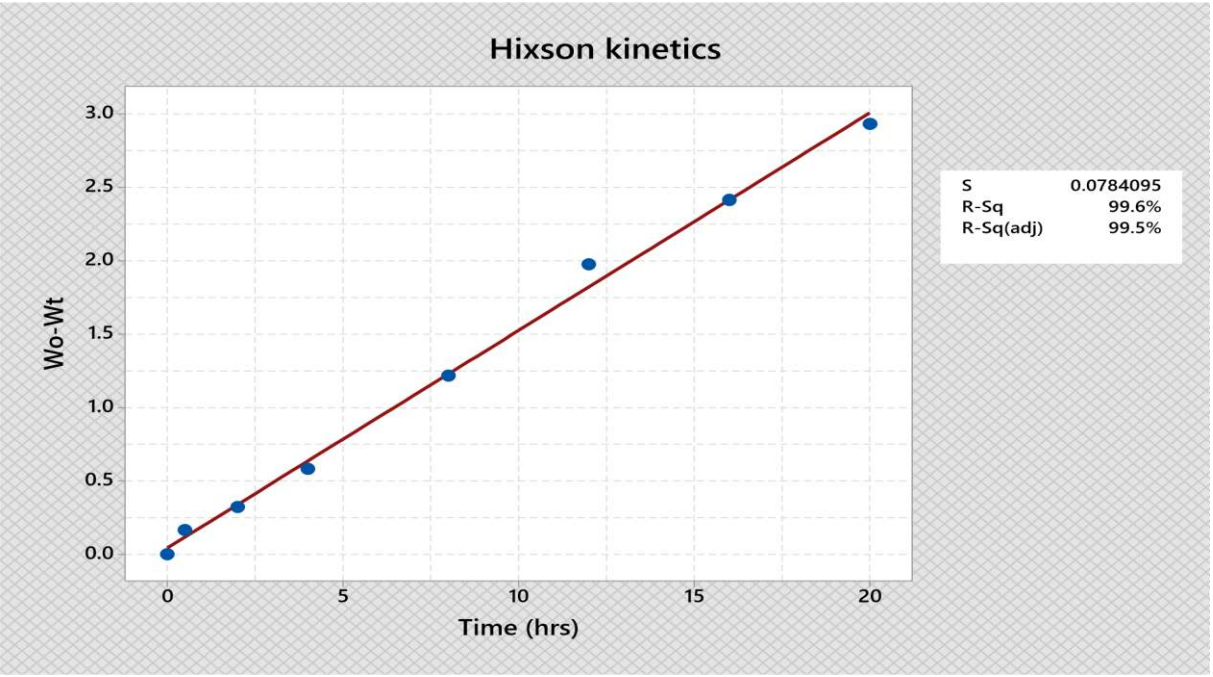
Graph 18 : First order drug release mechanism of optimized formulation SMVF-08

Higuchi model



Graph 19 : Higuchi model kinetics for optimized formulation SMVF-08

Hixson kinetic model

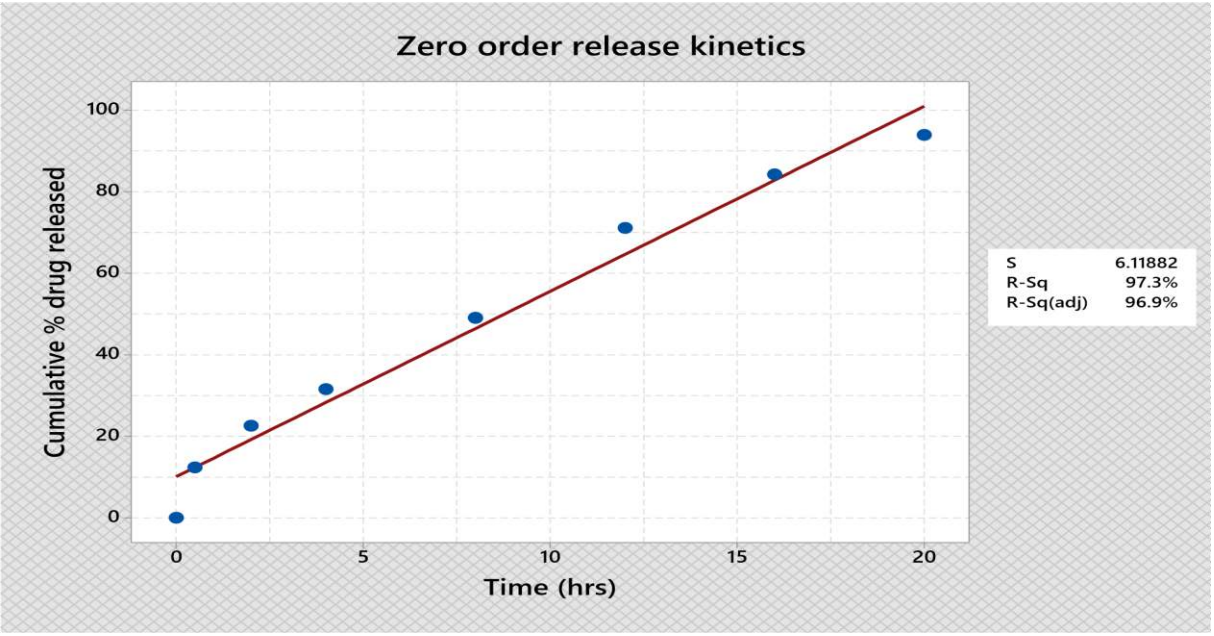


Graph 20 : Hixson model kinetics for optimized formulation SMVF-08

Table 19 :Determination of drug release mechanism of optimized formulation SMVF-12

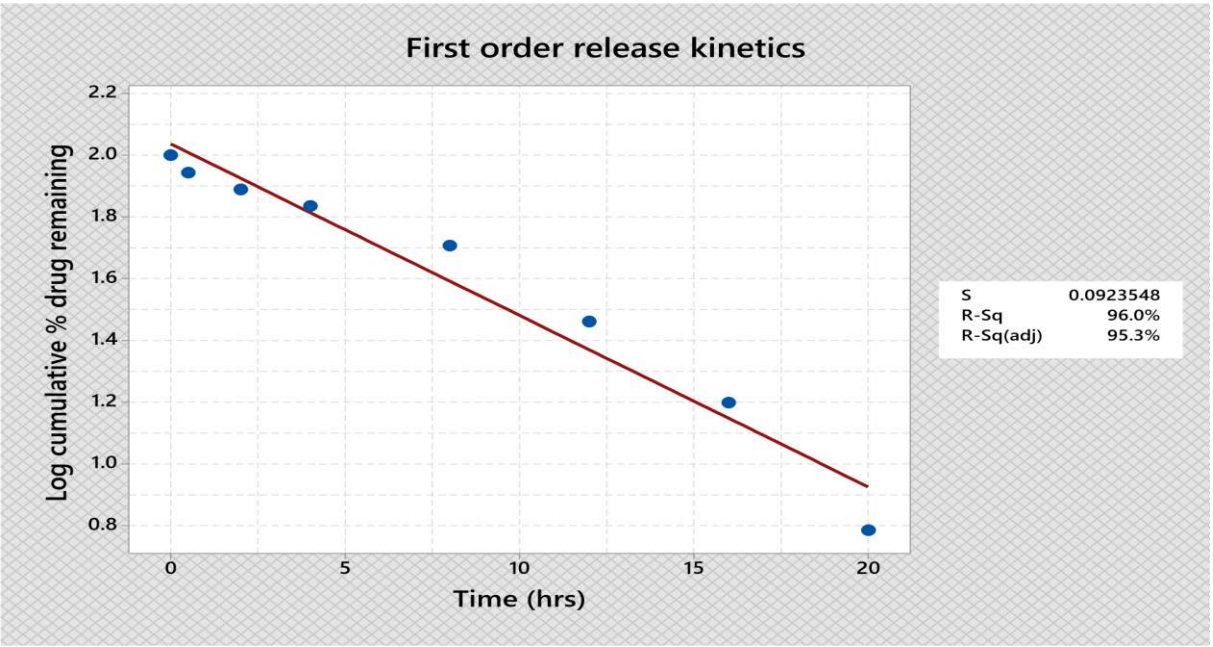
Time (in hrs)	cumulative percentage drug release	Percentage drug remain	Square root time	Log Cumulative percentage drug remaining	Log time	Log Cumulative percentage drug released	Percentage drug released	Cube Root of % drug Remaining (Wt.)	Wo-Wt.
0	0	100	0.000	2.000	0.000	0.000	0	4.642	0.000
0.5	12.37	87.63	0.707	1.943	-0.301	1.092	12.37	4.442	0.200
2	22.58	77.42	1.414	1.889	0.301	1.354	10.21	4.262	0.380
4	31.55	68.45	2.000	1.835	0.602	1.499	8.97	4.091	0.551
8	49.03	50.97	2.828	1.707	0.903	1.690	17.48	3.708	0.934
12	71.08	28.92	3.464	1.461	1.079	1.852	22.05	3.069	1.573
16	84.22	15.78	4.000	1.198	1.204	1.925	13.14	2.508	2.133
20	93.9	6.1	4.472	0.785	1.301	1.973	9.68	1.827	2.814

Zero order



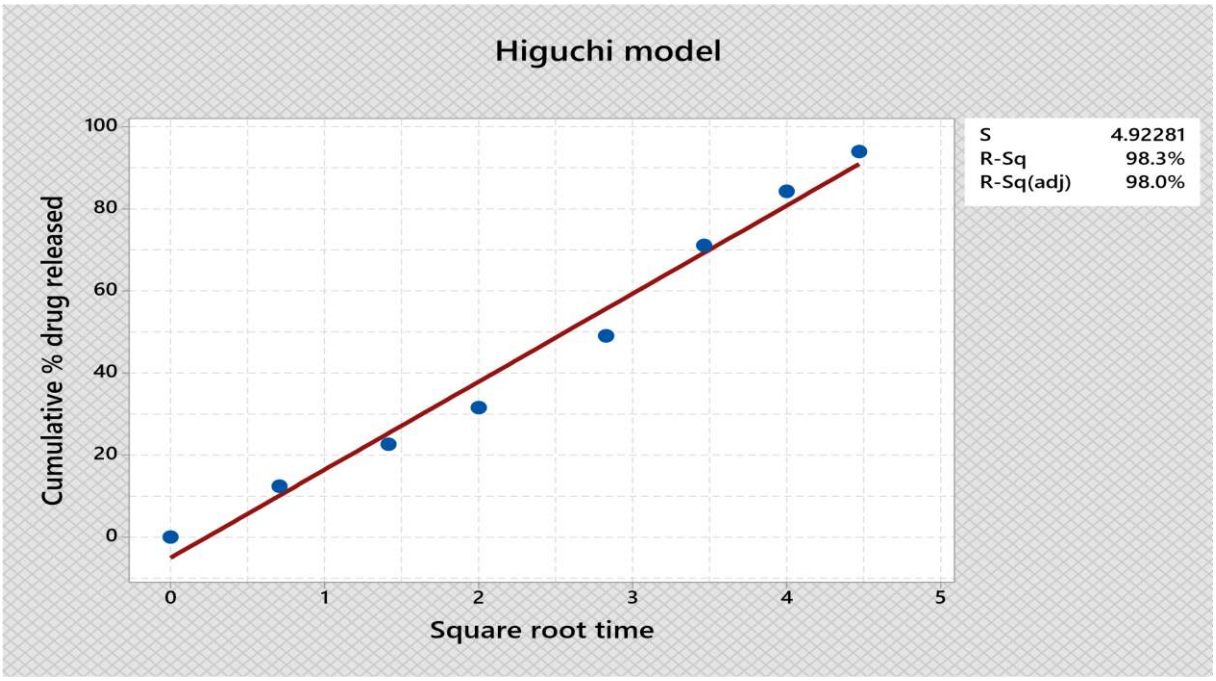
Graph 21 : Zero order drug release of optimized formulation SMVF-12

First order



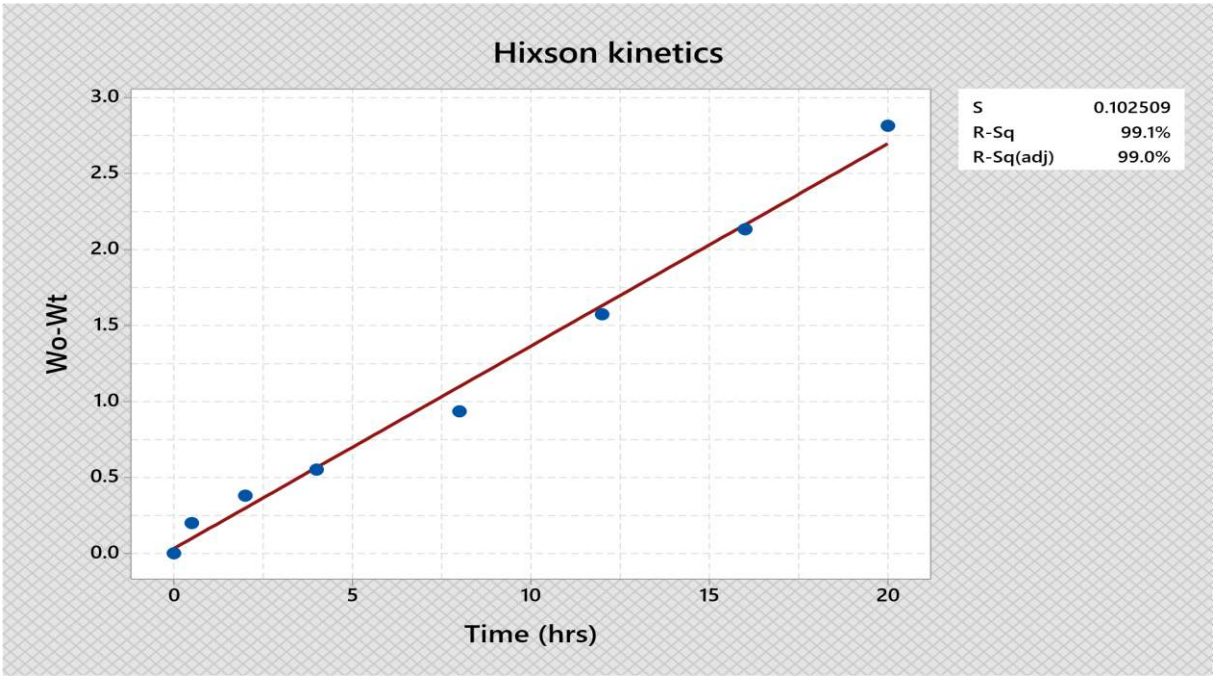
Graph 22 : First order drug release mechanism of optimized formulation SMVF-12

Higuchi model



Graph 23 : Higuchi model kinetics for optimized formulation SMVF-12

Hixson kinetic model

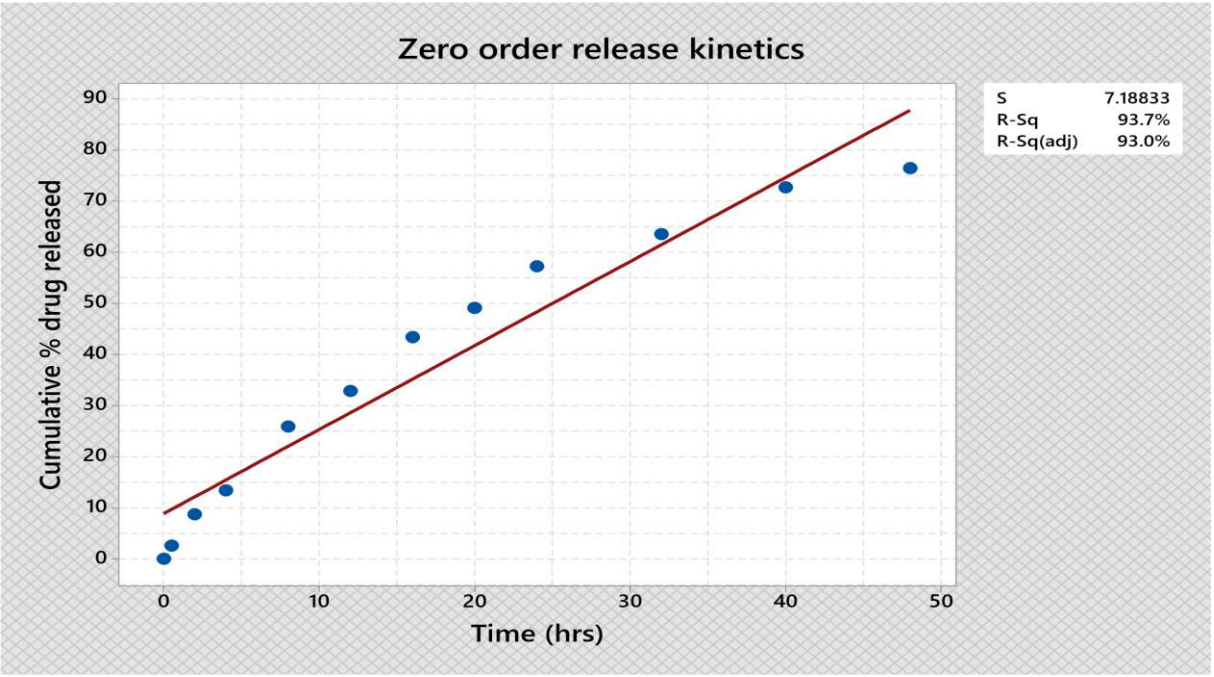


Graph 24 : Hixson model kinetics for optimized formulation SMVF-12

Table 20 : Determination of drug release mechanism of optimized formulation SMVF-16

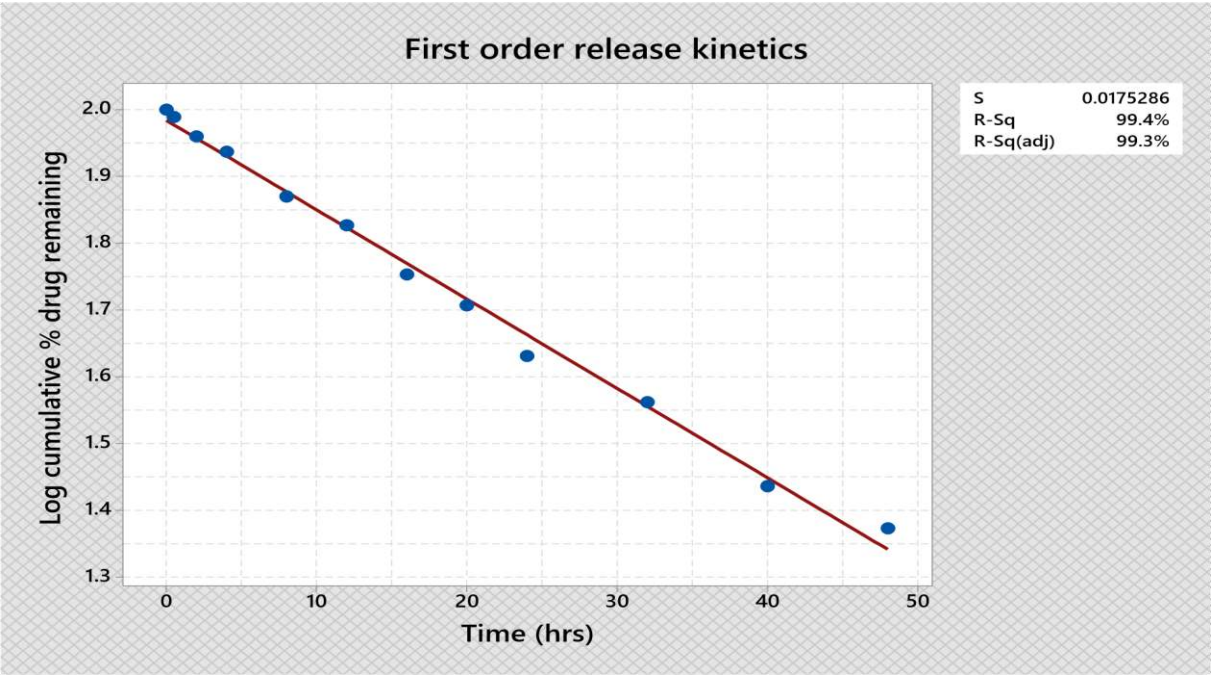
Time (in hrs)	Cumulative percentage drug release	Percentage drug remain	Square root time	Log Cumulative percentage drug remaining	Log time	Log Cumulative percentage drug released	Percentage drug released	Cube Root of % drug Remaining (Wt.)	Wo-Wt.
0	0	100	0.000	2.000	0.000	0.000	0	4.642	0.000
0.5	2.6	97.4	0.707	1.989	-0.301	0.415	2.60	4.601	0.041
2	8.71	91.29	1.414	1.960	0.301	0.940	6.11	4.503	0.139
4	13.42	86.58	2.000	1.937	0.602	1.128	4.71	4.424	0.218
8	25.9	74.1	2.828	1.870	0.903	1.413	12.48	4.200	0.442
12	32.87	67.13	3.464	1.827	1.079	1.517	6.97	4.064	0.578
16	43.39	56.61	4.000	1.753	1.204	1.637	10.52	3.840	0.801
20	49.08	50.92	4.472	1.707	1.301	1.691	5.69	3.706	0.935
24	57.26	42.74	4.899	1.631	1.380	1.758	8.18	3.496	1.145
32	63.55	36.45	5.657	1.562	1.505	1.803	6.29	3.316	1.325
40	72.68	27.32	6.325	1.436	1.602	1.861	9.13	3.012	1.629
48	76.41	23.59	6.928	1.373	1.681	1.883	3.73	2.868	1.773

Zero order



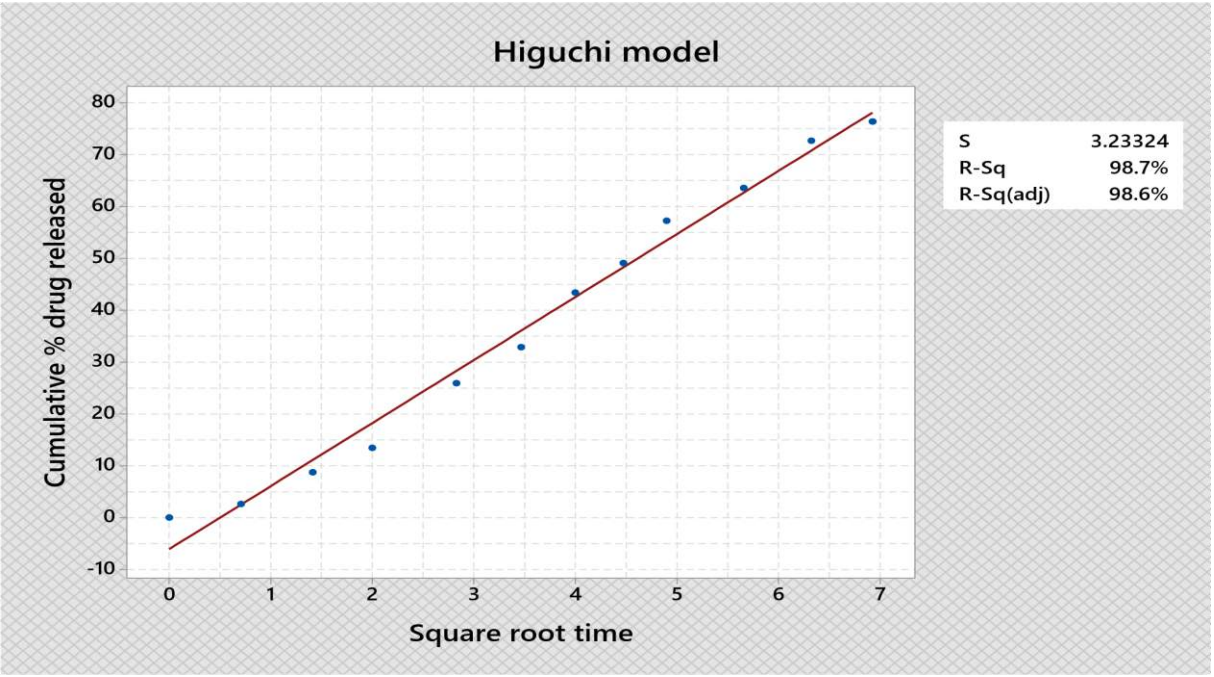
Graph 25 : Zero order drug release of optimized formulation SMVF-16

First order



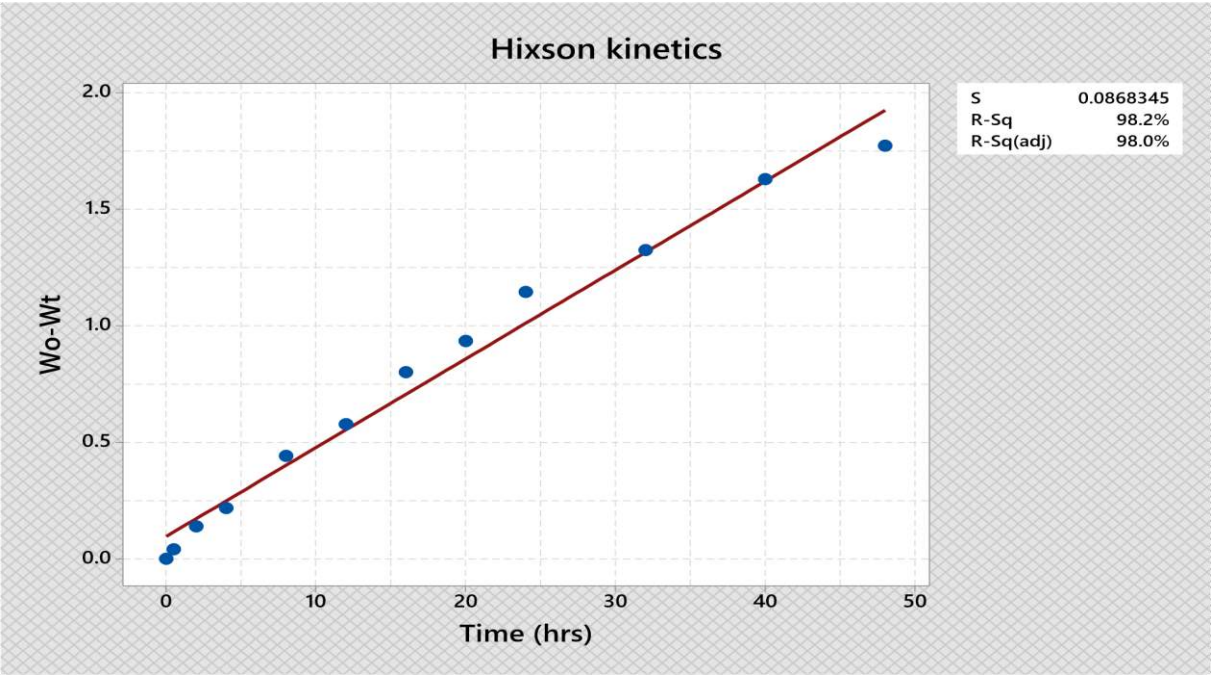
Graph 26 : First order drug release of optimized formulation SMVF-16

Higuchi model



Graph 27 : Higuchi model kinetics for optimized formulation SMVF-16

Hixson kinetic model

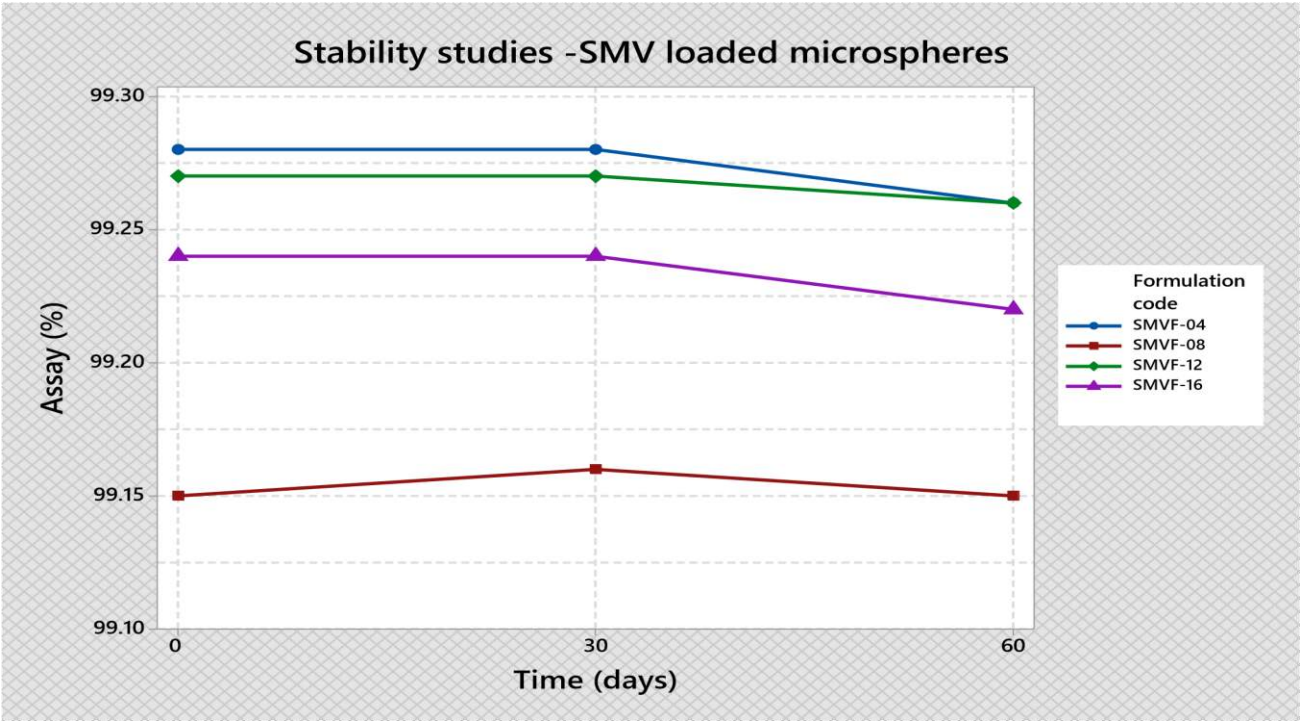


Graph 28 : Hixson model kinetics for optimized formulation SMVF-16

Stability Studies

The prepared and optimized formulation loaded for Stability study to evaluate the storage and shelf life prediction. Stability studies was carried out in accelerated stability chamber 40°C / 75% RH for a period of 60 days. The results was drawn below.

Time points (in days)	SMV loaded HPMC microspheres	SMV loaded Ethyl cellulose microspheres	SMV loaded Carbopol microspheres	SMV loaded PLGA microspheres
0 (initial)	99.28	99.15	99.27	99.24
30	99.28	99.16	99.27	99.24
60	99.26	99.15	99.26	99.22



Graph 29 : Stability study data

DISCUSSION

Essential of the present study was to prevent extensive metabolism of the drug and consequently to enhance the oral bioavailability of the drug in the form of controlled release (long acting) microspheres .Attempt has been made to fabricate controlled release microspheres of Simvastatin, a BCS class-II drug with low solubility. The microspheres were prepared by solvent evaporation method using various class of polymers to retard the drug release and the process parameters were augmented.

Rendering to the results of FT-IR analysis, no drug-excipient interaction/incompatibility occurred with the polymers and API. Stability study of formulation exhibits good consistency with the respective Accerlated and Real time storage conditions.

The yield of formulation observed with higher yield in SMVF-16, SMVF-08,SMVF-12, & SMVF-03 formulation yield range of 96.85%,96.60%,95.53% & 89.20% respectively.

Microspheres morphology was evaluated by Scanning Electron Microscope (SEM).SEM Photographs of optimized formulation SMVF-04, SMVF-08, SMVF-12 and SMVF-16 exhibits distinct, spherical shapes with good morphological characteristics.

Entrapment efficiency was significantly higher with PLGA and Carbopol 940 microspheres when compared to Ethyl cellulose containing microspheres.HPMC containing shown lower entrapment efficiency due to higher in viscosity. High entrapment of Simvastatin was occurred due to its poor aqueous solubility, high binding of drug & polymer in organic phase and increased polymer ratio.

An increase in polymer concentration drug loading efficiency was markedly enhanced. This was possibly caused by hydrophobic nature of drug and poor dispersibility of polymers into the aqueous phase. Drug content analysis results exhibits the extreme drug loading in SMVF-16,SMVF-12,SMVF-08 & SMVF-04 range of 34.76,32.47,33.32 & 30.61 mg respectively.

When the polymer concentration increases, leads to increase in the viscosity of the polymer matrix and which thus results in decrease the diffusion coefficient of the drug and reduction in the release rate of the drug. For the higher viscosity, they become more resistant to dilution and erosion. Drug release from the prepared microsphere formulation exhibited the controlled release for prolonged time due to release mechanism of polymers.

The duration and percentage of drug released drawn below 16 hrs for HPMC microspheres (94.29%) -SMVF-04,20 hrs for EC microspheres (94.90%) -SMVF-08, 32 hrs for Carbopol 940 microspheres (92.80%) -SMVF-12 & 48 hrs for PLGA microspheres (86.41%) -SMVF-16.

Drug release mechanism was performed through pharmacokinetic study from the drug product by using zero order, First order, Higuchi's model and Hixson model. From the obtained data with kinetic study R^2 values (regression coefficient) was calculated. The order of release was found to be zero order, in which R^2 value was closer to 1.0.

Hence the formulation follows zero order kinetics and probably follows higuchi model. From the obtained values concluded that formulation containing polymers are releasing drug via diffusion, swelling controlled and matrix erosion mechanism.

CONCLUSION

In this work only Physico-chemical characterization and in-vitro evaluation of Simvastatin with various class of polymers were performed.

From the in-vitro and stability studies its concluded for a short-term release requirement upto 16 hrs HPMC polymer loaded simvastatin microspheres can be recommended. For intermediate drug release over a period time of 24 hrs Ethyl cellulose loaded simvastatin microspheres can be recommended. For long term requirement of therapeutic need its promised with Carbopol 940 and PLGA microspheres (upto 48 hrs).The controlled release behavior attained with the aid of sodium alginate which is incorporated to the formulation to provide the controlled release.

The prepared formulation of microsphere is designed to administer via tablet by compression, capsule filling method and injectable formulation (with expected particle size).

Based on the above mentioned reasons such microsphere drug delivery system is presently need for successful drug delivery in severe atherosclerotic /hyperlipidemic patients. Further studies can be performed to develop the product extreme consumption of commercial usage.

Future perspectives

So, in future in-vivo release study by using different models are obliged to set the in vitro and in-vivo correlation (IVIVC) which is essential for development and long term stability studies are prescribed of successful formulation.

Along with predictive mathematical model describing relationship between an in-vitro property of a dosage form and an in-vivo response to be established to prove the consistency of formulation.
Prolonged release of anti-lipidemic drug from microsphere formulation expected to maintain the plasma concentration of drugs in between minimum inhibitory concentration and maximum safe concentration (therapeutic concentration) to be proven.

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