DEVELOPMENT AND CHARACTERIZATION OF SIMVASTATIN LOADED CHITOSAN NANOPARTICLES FOR SUSTAINED DRUG DELIVERY

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
PHARMACEUTICS

Submitted by
Reg. No. 261211257
Under the Guidance of

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COLLEGE OF PHARMACY
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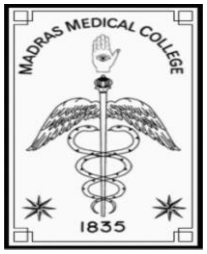
APRIL 2014



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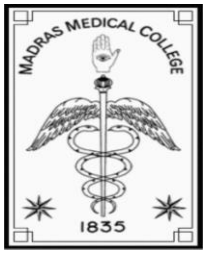

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Place: Chennai-03.

Date: (Dr. A.Jerad Suresh)

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Place: Chennai-03.

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CERTIFICATE

This is to certify that Miss. R. Rajakumari, Post Graduate student, Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03 had submitted her protocol (Part B application) vide. 9/243/CPCSEA for the dissertation Programme to the Animal Ethical Committee, Madras Medical College, Chennai-03.

TITLE

DEVELOPMENT AND CHARACTERIZATION OF SIMVASTATIN LOADED CHITOSAN NANOPARTICLES FOR SUSTAINED DRUG DELIVERY.

The Animal Ethical Clearance Committee experts screened her proposal No: vide. 9/243/CPCSEA and have given clearance in the meeting held on 22.11.13 at Dean's Chamber in MMC, Chennai-03. Her study involves only small animals of house dwelling rats.

17.2.14.

Signature

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ACKNOWLEDGEMENT

"Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow"

I consider this as an opportunity to express my gratitude to all the dignitaries who have been involved directly or indirectly with the successful completion of this dissertation. The satisfaction that accompanies the successful completion of any task would be incomplete without mention of the people who made it possible with constant guidance, support and encouragement that crows all effort with success.

Many Thanks to **ALMIGHTY GOD**, for it, He who began this work in me and carried it to completion. It is He who has blesses me with the people whose names I feel privileged to mention here.

I acknowledge my sincere thanks to **Prof. Dr. A. Jerad Suresh M.Pharm., Ph.D., MBA**, Principal, College of Pharmacy, Madras Medical College, Chennai, for his continuous support in carrying out my project work in this institution.

It is with great pleasure that I place on record a deep sense of gratitude and Heartfelt thanks to my guide **Mr.K.Elango, M.Pharm.,(Ph.D.),** Head, Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai, for their help, support and constant encouragement throughout the progress of this work. It was really a great experience working under them and their guidance, which was of immense help in my project work without which it would have been an unachievable task.

It's a great pleasure for me to acknowledge my sincere thanks to all my teaching staff members **Dr. N.Deattu, M.Pharm., Ph.D, Mrs. S.Daisy Chellakumari, M.Pharm., Mrs. R.Devi Damayanthi, M.Pharm**., of the Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03.

I extent my thanks to all non-teaching staff members **Mr.R.Marthandam** and **Mrs.R.Shankari** Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03.

The words are insufficient to thank my mentors **Vengatesh.S, Pasupathi Raja. M and Sivasubramani.R.V** and who stood beside me each and every step during my project and given me constant support.

I have no words to express my pleasure in thanking my dear friends **David selva Kumar.D**, **Chinnaraja.S, Ramu.P, Kishore kumar.S, Catherin.U, Priya.B, Akilandeshwari.A, Suhasini.G** and all others who are behind me supporting my endeavour.

I extend my cordial thanks to my seniors and to my juniors for their kind support and co-operation.

Most of all I would like to thank my beloved parents, brother, and my dearest friends for their priceless support, love and encouragement throughout the entire tenure of this course.

DEDICATED TO MY FAMILY & MY PROFESSION

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List of Abbreviations

AI	Atherogenic Index
CS	Chitosan
CMC	Carboxy Methyl Cellulose
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infra-Red Spectroscopy
LB	Libermann- Burchard
LDL	Low Density Lipoproteins
PCS	Photon Correlation Spectroscopy
PNPs	Polymeric Nanopaarticles
PTA	Phospho Tungstic Acid
TC	Total Cholesterol
TG	Triglycerides
SEM	Scanning Electron Microscopy
VLDL	Very Low Density Lipoproteins
XRD	X-Ray Differaction
ml	millilitre
µg	Microgram
mg	milligram
nm	nanometer

INTRODUCTION

1. INTRODUCTION

1.1 DRUG DELIVERY SYSTEM[1]:

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

- As bringing new drug entities into the market is an expensive and time consuming process, development of new drug delivery is profitable.
- New system is needed to deliver novel, genetically engineered pharmaceuticals such as protein and peptides to their sites of action without biological inactivation.
- To improve therapeutically efficacy and safety of conventional drug both by reducing size and number of doses.

Two important features are important while developing a drug delivery system. i.e. It should deliver drug at a rate dictated by needs of body over the entire period of treatment and the drug should solely reach the site of action.

1.2 NOVEL DRUG DELIVERY SYSTEM [(2, 3)]

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins,

liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Controlled drug release and subsequent biodegradation are important for developing successful formulations. Potential release mechanisms involve: (i) desorption of surface-bound /adsorbed drugs; (ii) diffusion through the carrier matrix; (iii) diffusion (in the case of nanocapsules) through the carrier wall; (iv) carrier matrix erosion; and (v) a combined erosion /diffusion process. The mode of delivery can be the difference between a drug's success and failure, as the choice of a drug is often influenced by the way the medicine is administered. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature).

For over 20 years, researchers have appreciated the potential benefits of nanotechnology in providing vast improvements in drug delivery and drug targeting. Improving delivery techniques that minimize toxicity and improve efficacy offers great potential benefits to patients, and opens up new markets for pharmaceutical and drug delivery companies. Other approaches to drug delivery are focused on crossing particular physical barriers, such as the blood brain barrier, in order to better target the drug and improve its effectiveness; or on finding alternative and acceptable routes for the delivery of protein drugs other than via the gastro-intestinal tract, where degradation can occur.

Drug Delivery Carriers

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as drug delivery systems. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity. The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possesses amphiphilic and/or mesogenic properties.

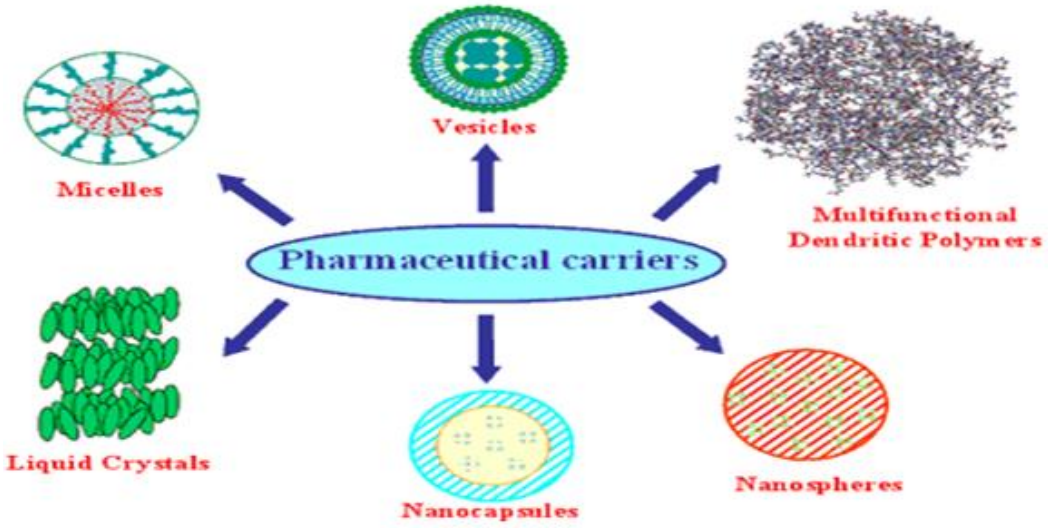


Fig.1: Schematic representation of various Pharmaceutical carriers

Nanotechnology[4]

Nanotechnology is science of matter and material that deal with the particle size in nanometers. The word' nano' is derived from latin word, which means dwarf ($1nm=10^{-9}m$). Nanomedicine deals with comprehensive monitoring, control, construction, repair, defense and improve human biological system at molecular level using engineered nanostructures and nanodevices.

Pharmaceutical nanotechnology embraces applications of nanoscience to pharmacy as nanomaterials, and as devices like drug delivery, diagnostic, imaging and biosensor materials. Pharmaceutical nanotechnology has provided more fine-tuned diagnosis and focused

treatment of disease at a molecular level. It helps in detecting the antigen associated with diseases such as cancer, diabetes mellitus, neuro degenerative diseases, as well as detecting the microorganisms and virus associated with infections. In pharmacy size reduction has an important application as drugs in the nanometre size range enhance performance in a variety of dosage forms.

ADVANTAGES OF NANOPARTICLES

1. Increased surface area.
2. Enhanced solubility.
3. Increased rate of dissolution.
4. Increased in oral bioavailability.
5. Less amount of dose required & reduces the number of doses.
6. Protection of drug from degradation.
7. More rapid onset of therapeutic action.
8. Achievement of drug targeting.
9. Passive targeting of drugs to the macrophages present in the liver and Spleen.
10. Increased efficacy and therapeutic index.
11. Increased stability via encapsulation.
12. Improved pharmacokinetic effect.
13. Entrap both hydrophilic & lipophilic drug Protect entrapped drug from enzymatic degradation.
14. Large variety of drugs (antineoplastic, antibiotic), peptides or protein (including antibodies), viruses and bacteria can be incorporated into nanoparticles.
15. Nanoparticles encapsulated drugs are delivered intact to various tissue and cells and can be released when nanoparticles are destroyed ,enabling site specific and targeted drug delivery.
16. Other tissues and cells of the body are protected from drug until it is released by nanoparticles thus decreasing drug toxicity.

Polymers used in Nanoparticle

Polymeric Nanoparticles[5]

The polymeric nanoparticles (PNPs) are prepared from biocompatible and biodegradable polymers in size between 10-1000 nm where the drug is dissolved, entrapped,

encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed[6,7]. The field of polymer nanoparticles (PNPs) is quickly expanding and playing an important role in a wide spectrum of areas ranging from electronics, photonics, conducting materials, sensors, medicine, biotechnology, pollution control and environmental technology[8-16]. PNPs are promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to specific target, such an advantage improves the drug safety[17]. Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometre-size promotes effective permeation through cell membranes and stability in the blood stream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into unique nanoparticle constructs with many potential medical applications[18]. Several methods have been developed during the last two decades for preparation of PNPs, these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer or ionic gelation method.

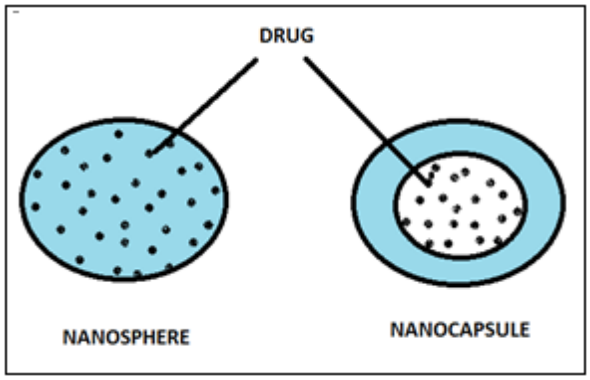


Fig.2: Difference between the nanosphere and nanocapsule

Advantages of polymeric nanoparticles [19, 20.]

- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.

- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

Polymers used in preparation of nanoparticles

Characteristic features of polymers[21].

A polymer used in controlled drug delivery formulations, must be:

- Chemically inert
- Non-toxic
- Free of leachable impurities
- An appropriate physical structure
- With minimal undesired aging
- Readily processable

Classification:

Polymers are classified as[22-25]

A) Natural polymers:

- Gums (Ex. Acacia, Guar, etc.)
- Chitosan
- Gelatin
- Sodium alginate
- Albumin

B) Synthetic polymers:

a) Nonbiodegradable:

- Cellulosics
- Poly(2-hydroxy ethyl methacrylate)
- Poly (N-vinyl pyrrolidone)
- Poly(methyl methacrylate)
- Poly (vinyl alcohol).
- Poly (acrylic acid).

- Polyacrylamide.
- Poly (ethylene-co-vinyl acetate).
- Poly (ethylene glycol).
- Poly (methacrylic acid).

b) Biodegradable

- Polylactides (PLA).
- Polyglycolides (PGA).
- Poly (lactide-co-glycolides) (PLGA).
- Polyanhydrides.
- Polyorthoesters.
- Polycyanoacrylates
- Polycaprolactone

Originally, polylactides and polyglycolides were used as absorbable suture material. The main advantage of these degradable polymers is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways. However, biodegradable materials do produce degradation by-products that must be tolerated with little or no adverse reactions within the biological environment.

Mechanism of drug release[21]

The polymeric drug carriers deliver the drug at the tissue site by any one of the three general physicochemical mechanisms.

1. By the swelling of the polymer nanoparticles by hydration followed by release through diffusion.

2. By an enzymatic reaction resulting in rupture or cleavage or degradation of the polymer at site of delivery, there by releasing the drug from the entrapped inner core.

3. Dissociation of the drug from the polymer and its de-adsorption/release from the swelled nanoparticles.

Techniques of preparation

The properties of PNPs have to be optimized depending on the particular application. In order to achieve the properties of interest, the mode of preparation plays a vital role. Thus, it is highly advantageous to have preparation techniques at hand to obtain PNPs with the desired properties for a particular application. Different techniques like polymerization, preformed polymers or ionic gelation etc., are used.

Methods for preparation of nanoparticles from dispersion of preformed polymer

Dispersion of drug in preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA), poly (D, L-glycolide) (PLG), poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA). These can be accomplished by different methods described below.

a) Solvent evaporation

b) Nanoprecipitation

c) Emulsification/solvent diffusion

d) Salting out

e) Dialysis

f) Supercritical fluid technology (SCF)

Methods for preparation of nanoparticles from polymerization of monomers

a) Emulsion

b) Mini emulsion

c) Micro emulsion

d) Interfacial polymerization

e) Controlled/Living radical polymerization(C/LRP)

Ionic gelation or coacervation of hydrophilic polymers

Solvent evaporation

Solvent evaporation was the first method developed to prepare PNPs. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, dichloromethane and chloroform preformed polymer were widely used, but are now replaced with ethyl acetate which has a better toxicological profile. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. In the conventional methods, two main strategies are being used for the formation of emulsions, the preparation of single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These

methods utilize high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized.[26, 27] Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

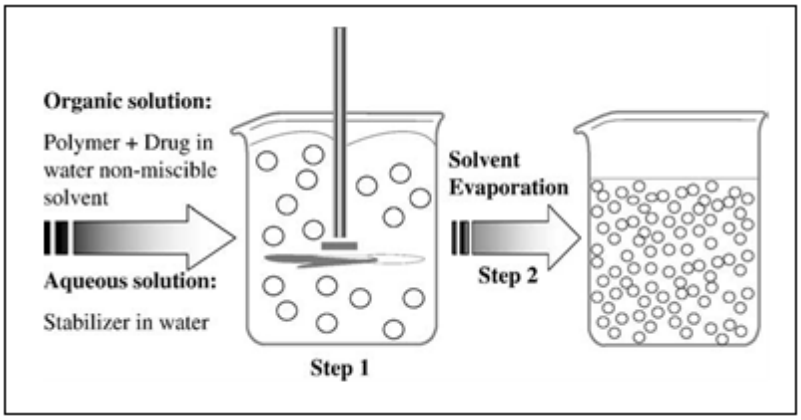


Fig.3: Schematic representation of the solvent-evaporation technique[27]

Nanoprecipitation

Nanoprecipitation is also called solvent displacement method. It involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant[28-31]. The polymer generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension. To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent that is also a non-solvent of the polymer. The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared. The usefulness of this simple technique[32] is limited to water-

miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. Then, even though some water-miscible solvents produce a certain instability when mixed in water, spontaneous emulsification is not observed if the coalescence rate of the formed droplets is sufficiently high[33]. Although, acetone/dichloromethane (ICH, class 2) are used to dissolve and increase the entrapment of drugs, the dichloromethane increases the mean particle size[34] and is considered toxic. This method is basically applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase, and it is not an efficient means to encapsulate water-soluble drugs. This method has been applied to various polymeric materials such as PLGA, PLA, PCL, and poly (methyl vinyl ether-comaleic anhydride) (PVM/MA). This technique was well adapted for the incorporation of cyclosporin A, because entrapment efficiencies as high as 98% were obtained. Highly loaded nanoparticulate systems based on amphiphilic h-cyclodextrins to facilitate the parenteral administration of the poorly soluble antifungal drugs Bifonazole and Clotrimazole were prepared according to the solvent displacement method

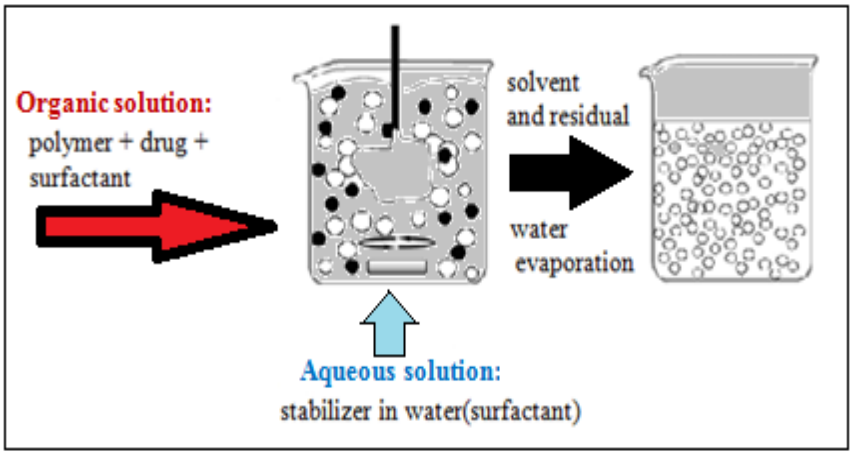


Fig.4 Schematic representation of the nanoprecipitation technique. Surfactant is optional.

Emulsification/solvent diffusion (ESD)

This is a modified version of solvent evaporation method. The encapsulating polymer is dissolved in a partially water soluble solvent such as propylene carbonate and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. In fact, to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partly miscible with water or with another organic solvent

in the opposite case. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point.This technique presents several advantages, such as high encapsulation efficiencies (generally >70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency[7,27]. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs[32]. Several drug-loaded nanoparticles were produced by the ESD technique, including mesotetra(hydroxyphenyl)porphyrin-loaded PLGA (p-THPP) nanoparticles, doxorubicin-loaded PLGA nanoparticles, plasmid DNA-loaded PLA nanoparticles, coumarin-loaded PLA nanoparticles, indocyanine, cyclosporine (Cy-A)-loaded gelatin and cyclosporin (Cy-A)-loaded sodium glycolate nanoparticles

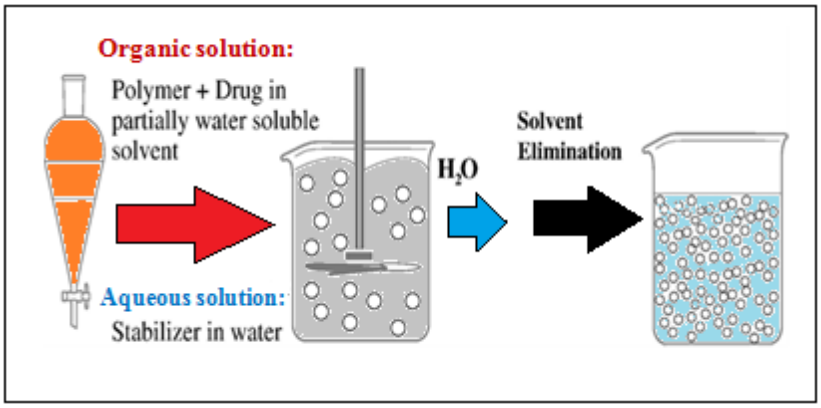


Fig.5: Schematic representation of the emulsification/solvent diffusion technique

Salting out

Salting out is based on the separation of a water miscible solvent from aqueous solution via a salting out effect. The salting out procedure can be considered as a modification of the emulsification/solvent diffusion. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non- electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a

sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres[27]. The selection of the salting out agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting out agent are then eliminated by cross-flow filtration. This technique used in the preparation of PLA, poly (methacrylic) acid, nanospheres leads to high efficiency and is easily scaled up. The main advantage of salting out is that it minimizes stress to protein encapsulants[35]. Salting out does not require an increase of temperature and therefore, may be useful when heat sensitive substances have to be processed[36]. The greatest disadvantages are exclusive application to lipophilic drugs and the extensive nanoparticle washing steps[37].

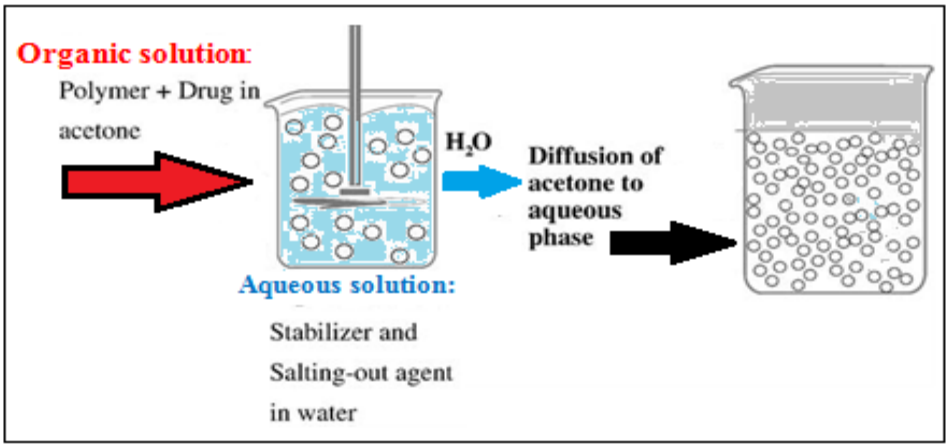


Fig.6: Schematic representation of the Salting out technique

Preparation of nanoparticles by polymerization of a monomer

To attain the desired properties for a particular application, suitable polymer nanoparticles must be designed, which can be done during the polymerization of monomers. Processes for the production of PNPs through the polymerization of monomers are discussed below.

Emulsion polymerization

Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable. The method is classified into two categories, based on the use of an organic or aqueous continuous phase. The continuous organic phase methodology involves the dispersion of monomer into an emulsion or inverse microemulsion, or into a material in which the monomer is not soluble (nonsolvent)[27]. Polyacrylamide nanospheres were produced by this method.As one of the first methods for production of nanoparticles,

surfactants or protective soluble polymers were used to prevent aggregation in the early stages of polymerization. This procedure has become less important, because it requires toxic organic solvents, surfactants, monomers and initiator, which are subsequently eliminated from the formed particles. As a result of the non-biodegradable nature of this polymer as well as the difficult procedure, alternative Approaches are of greater interest. Later, poly (methylmethacrylate) (PMMA), poly (ethylcyanoacrylate) (PECA), and poly(butylcyanoacrylate) nanoparticles were produced by dispersion via surfactants into solvents such as cyclohexane (ICH, class 2), n-pentane (ICH, class 3), and toluene (ICH, class 2) as the organic phase . In the aqueous continuous phase the monomer is dissolved in a continuous phase that is usually an aqueous solution, and the surfactants or emulsifiers are not needed. The polymerization process can be initiated by different mechanisms. Initiation occurs when a monomer molecule dissolved in the continuous phase collides with an initiator molecule that might be an ion or a free radical. Alternatively, the monomer molecule can be transformed into an initiating radical by high-energy radiation, including g-radiation, or ultraviolet or strong visible light. Chain growth starts when initiated monomer ions or monomer radicals collide with other monomer molecules according to an anionic polymerization mechanism. Phase separation and formation of solid particles can take place before or after termination of the polymerization reaction.

Interfacial polymerization

It is one of the well-established methods used for the preparation of polymer nanoparticles. It involves step polymerization of two reactive monomers or agents, which are dissolved respectively in two phases (i.e., continuous- and dispersed-phase), and the reaction takes place at the interface of the two liquids. Nanometer-sized hollow polymer particles were synthesized by employing interfacial cross-linking reactions as polyaddition and polycondensation or radical polymerization. Oil-containing nanocapsules were obtained by the polymerization of monomers at the oil/water interface of a very fine oil-in-water micro-emulsion. The organic solvent, which was completely miscible with water, served as a monomer vehicle and the interfacial polymerization of the monomer was believed to occur at the surface of the oil droplets that formed during emulsification. To promote nanocapsule formation, the use of aprotic solvents, such as acetone and acetonitrile was recommended. Protic solvents, such as ethanol, n-butanol and isopropanol, were found to induce the formation of nanospheres in addition to nanocapsules. Alternatively, water-containing nanocapsules can be obtained by the interfacial polymerization of monomers in water-in-oil

micro-emulsions. In these systems, the polymer formed locally at the water-oil interface and precipitated to produce the nanocapsule shell.

Ionic gelation or coacervation of hydrophilic polymers

Polymeric nanoparticles are prepared by using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation.[38,39] The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a poly anion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

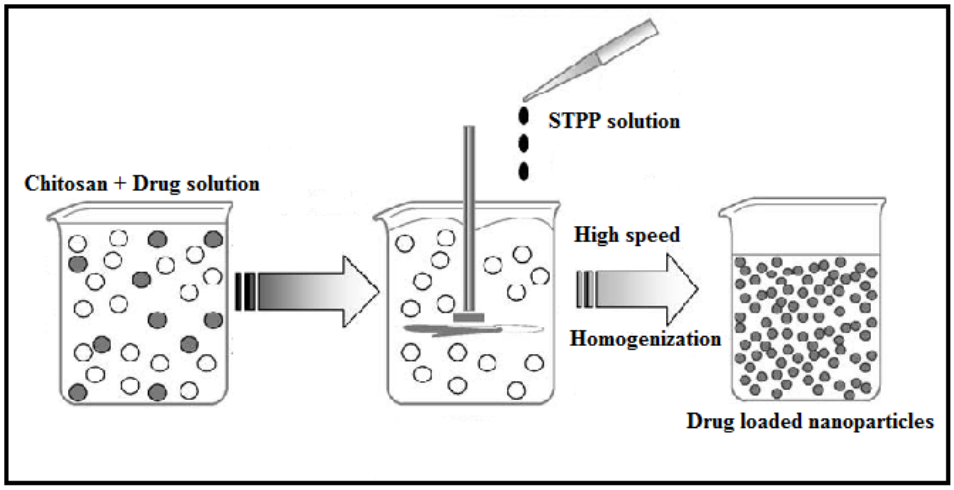


Fig.7: Schematic representation of ionic gelation method

CHARACTERIZTION OF NANO PARTICLES[4]

The nanoparticles are generally characterized for size, density, electrophoretic mobility, angle of contact and specific surface area. Table.1

PARAMETERS	CHARACTERISATION METHODS
Partical size and size distribution	Photon correlation spectroscopy (PCS), Laser defractrometry, Transmission electron microscopy, Scanning electron icroscopy(SEM), Atomic force microscopy(AFM), Mercury porositometry
Charge determination	Laser Doppler Anemometry, Zeta potentiometer
Surface hydrophobicity	Water contact angle measurements, Rose Bengal (dye) binding, Hydrophobic interaction chromatography, X-ray photoelectron spectroscopy
Chemical analysis of surface	Static secondary ion mass spectrometry, Sorptometer
Carrier drug interaction	FTIR, XRD, Differential Scanning Calorimetry
Nanoparticle dispersion stability	Critical flocculation temperature

Applications of Nanoparticulate Delivery Systems[7]

- Tumor targeting using nanoparticulate delivery systems
- Long circulating nanoparticles
- Reversion of multi drug resistance in tumor cells
- Nanoparticles for oral delivery of peptides and proteins
- Targetting of nanoparticles to epithelial cells in the GI tract using ligands
- Nanoparticles for gene delivery
- Nanoparticles for drug delivery into the brain

Future Opportunities and Challenges[2,3]

1. Nanoparticles and nanoformulations have already been applied as drug delivery systems with great success; and nanoparticulate drug delivery systems have still greater potential for many applications, including anti-tumour therapy, gene therapy, AIDS therapy, radiotherapy, in the delivery of proteins, antibiotics, vaccines and as vesicles to pass the blood-brain barrier.

2. Nanoparticles provide massive advantages regarding drug targeting, delivery release and with their additional potential to combine diagnosis and therapy, emerge as one of the major tools in nanomedicine. The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, improve drug loading, targeting, transport, release and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remains a major problem and improvements in biocompatibility obviously are a main concern of future research.

3. There are many technological challenges to be met, in developing the following techniques:

- Nano-drug delivery systems that deliver large but highly localized quantities of drugs to specific areas to be released in controlled ways;
- Controllable release profiles, especially for sensitive drugs;
- Materials for nanoparticles that are biocompatible and biodegradable;
- Architectures / structures, such as biomimetic polymers, nanotubes;
- Technologies for self-assembly;
- Functions (active drug targeting, on-command delivery, intelligent drug release devices/bio responsive triggered systems, self-regulated delivery systems, systems interacting with the body, smart delivery);
- Virus-like systems for intracellular delivery;
- Nanoparticles to improve devices such as implantable devices/nanochips for nanoparticle release, or multi reservoir drug delivery-chips;
- Nanoparticles for tissue engineering; e.g. for the delivery of cytokines to control cellular growth and differentiation, and stimulate regeneration; or for coating implants with nanoparticles in biodegradable polymer layers for sustained release;
- Advanced polymeric carriers for the delivery of therapeutic peptide/proteins (biopharmaceutics);
- And also in the development of: Combined therapy and medical imaging, for example, nanoparticles for diagnosis and manipulation during surgery (e.g. thermotherapy with magnetic particles);

- Universal formulation schemes that can be used as intravenous, intramuscular or peroral drugs
- Cell and gene targeting systems.
- User-friendly lab-on-a-chip devices for point-of-care and disease prevention and control at home.
- Devices for detecting changes in magnetic or physical properties after specific binding of ligands on paramagnetic nanoparticles that can correlate with the amount of ligand.
- Better disease markers in terms of sensitivity and specificity.

Sustained Release Drug Delivery System[40]

Over the Past 30 years, as the expense and complications involved in marketing new drug entities have increased, with concomitant recognition of the therapeutic advantages of Sustained drug delivery, greater attention is being paid on development of oral sustained release drug delivery systems. The goal in designing sustained release drug delivery system is to reduce the frequency of the dosing, reducing the dose & providing uniform drug delivery. So, Sustained release dosage form is a dosage form that releases one or more drugs continuously in predetermined pattern for a fixed period of time, either systemically or locally to specified target organ. Sustained release dosage forms provide better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery.

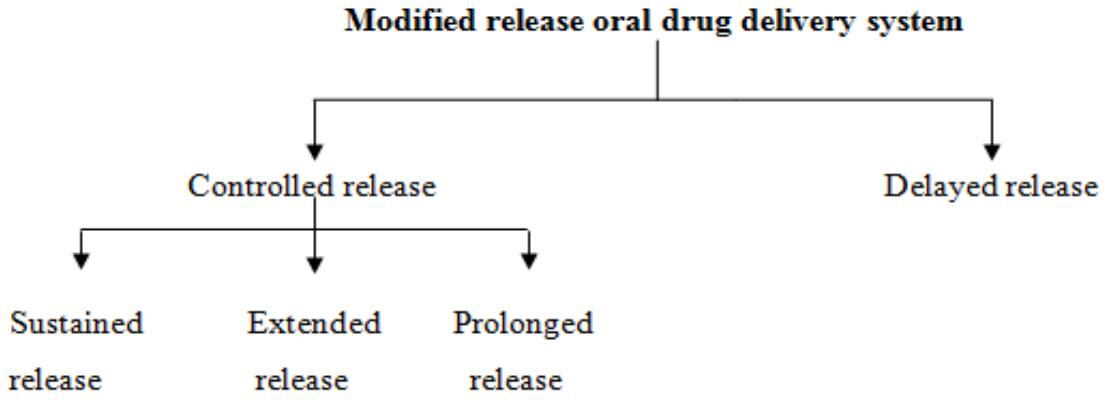
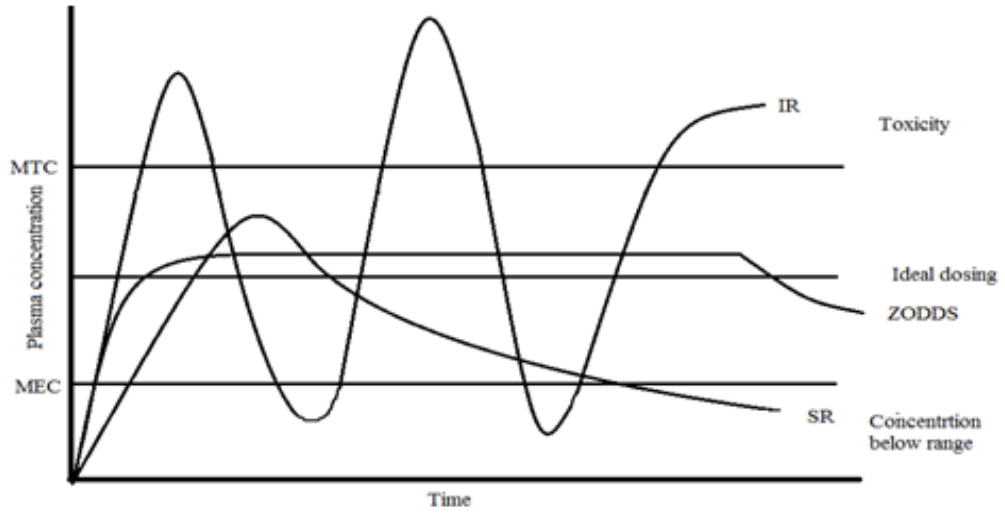


Fig.8: Classification of Modified Release Drug Delivery System

Sustained release drug delivery system

It includes any drug delivery system achieves release of drug over an extended period of time, which not depend on time. Hydrophilic polymer matrix is widely used for formulating a Sustained dosage form. The role of ideal drug delivery system is to provide proper amount of drug at regular time interval & at right site of action to maintain therapeutic range of drug in blood plasma.

The IR drug delivery system lacks some features like dose maintenance, sustained release rate & site targeting. The oral Sustained drug delivery has some potential advantage like Sustained release rate & dose maintenance in plasma. The SR formulations have some swelling polymer or waxes or both which controls the release rate. The use of reservoir system is also well known for controlling release rate.



Fg.9: Ideal Plasma Concentration Curves for Immediate Release, Zero Order Release, Sustained Release Drug Delivery System

ADVANTAGES OF SUSTAIN RELEASE DOSAGE FORMS[41]

1. Reduction in frequency of intakes.

2. Reduce side effects.

3. Uniform release of drug over time.

4. Better patient compliance

DISADVANTAGES OF SUSTAINED RELEASE DRUG DELIVERY

1. Increased cost.

2. Toxicity due to dose dumping.

3. Unpredictable and often poor *in vitro-in vivo* correlation.

4. Risk of side effects or toxicity upon fast release of contained drug (mechanical failure, chewing or masticating, alcohol intake).

5. Increased potential for first- pass clearance.

6. Need for additional patient education and counselling

PRINCIPLE OF SUSTAINED RELEASE DRUG DELIVERY[42]

The conventional dosage forms release their active ingredients into an absorption pool immediately. This is illustrated in the following simple kinetic scheme.

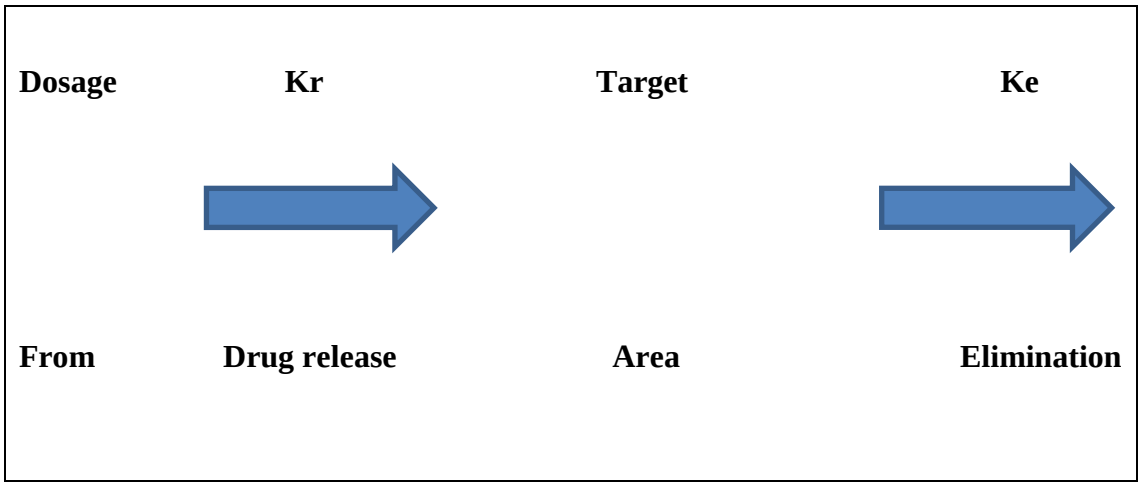


Fig.10: Conventional dosage form release

The absorption pool represents a solution of the drug at the site of absorption, and the term Kr, Ka and Ke are first order rate-constant for drug release, absorption and overall elimination respectively. Immediate drug release from a conventional dosage form implies that Kr>>>>Ka. Alternatively speaking the absorption of drug across a biological membrane is the rate-limiting step. For non-immediate release dosage forms, Kr<<<Ka i.e. the release of

drug from the dosage form is the rate limiting step. This causes the above Kinetic scheme to reduce to the following.

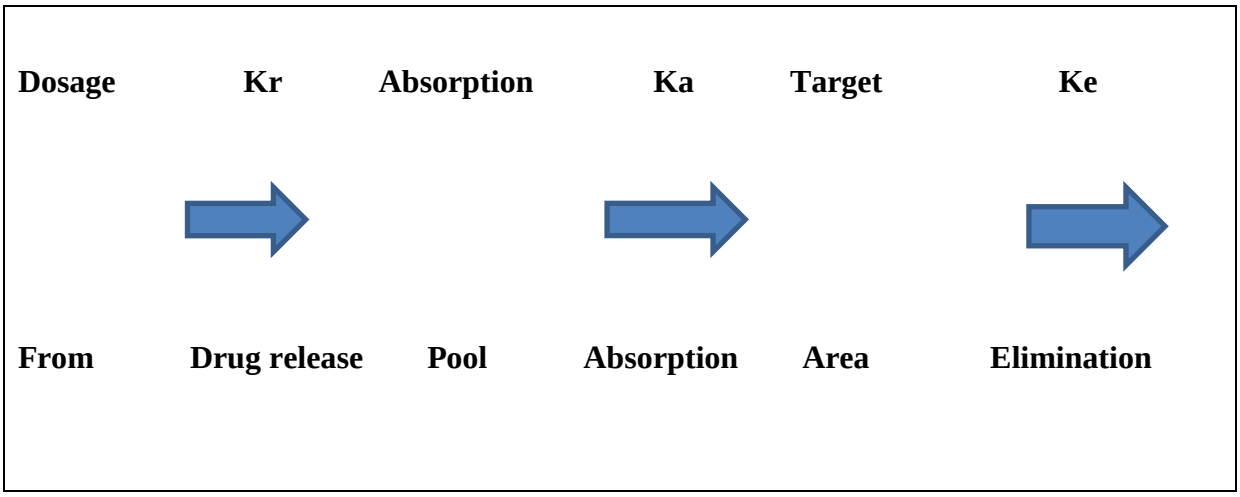


Fig.11: Sustained release

Essentially, the absorptive phase of the kinetic scheme become insignificant compared to the drug release phase. Thus, the effort to develop a non-immediate release delivery system must be directed primarily at altering the release rate. The main objective in designing a sustained release delivery system is to deliver drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be analogous to that achieved by continuous intravenous infusion where a drug is provided to the patient at a constant rate. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time. It means that the drug release from the dosage form should follows zero-order kinetics, as shown by the following equation:

$$Kr^{\circ} = \text{Rate in} = \text{Rate out} = Ke\ Cd\ Vd$$

Where,

Kr°= Zero-order rate constant for drug release-Amount/time

Ke = First-order rate constant for overall drug elimination time^{-1}

Cd= Desired drug level in the body - Amount/volume, and

Vd= Volume space in which the drug is distributed-Liters

The value of Ke, Cd and Vd are obtained from appropriately designed single dose pharmacokinetic study. The equation can be used to calculate the zero order release rate

constant. For many drugs, however, more complex elimination kinetics and other factors affecting their disposition are involved. This in turn affects the nature of the release kinetics necessary to maintain a constant drug blood level. It is important to recognize that while zero-order release may be desirable theoretically, non-zero-order release may be equivalent clinically to constant release in many cases. Sustained-release systems include any drug-delivery system that achieves slow release of drug over an extended period of time. If the systems can provide some control, whether this being of a temporal or spatial nature, or both, of drug release in the body, or in other words, the system is successful at maintaining constant drug levels in the target tissue or cells, it is considered a controlled-release system.

Formulation strategy for oral SRDDS[40]

- Diffusion sustained system
- Dissolution sustained system
- Methods using ion exchange
- Methods using osmotic pressure
- pH independent formulation
- Altered density formulation

Mechanism of drug release[41]

Diffusion is rate limiting Diffusion is driving force where the movement of drug molecules occurs from high concentration in the tablet to lower concentration in gastro intestinal fluids. This movement depends on surface area exposed to gastric fluid, diffusion pathway, drug concentration gradient and diffusion coefficient of the system

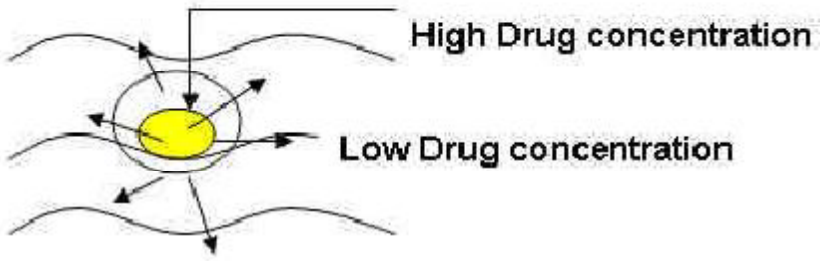


Fig.12: Mechanism of drug release

In practice, we can follow either of the two methods,

- The drug is formulated in an insoluble matrix; the gastric fluid penetrates the dosage form and dissolves the medicament and release the drug through diffusion.
- The drug particles are coated with polymer of defined thickness so as the portion of drug slowly diffuse through the polymer to maintain constant drug level in blood

LITERATURE REVIEW

2. LITERATURE REVIEW

Ambike *et al.,* [43] formulated the surface solid dispersions (SSD) of Simvastatin to improve the aqueous solubility and dissolution rate to facilitate faster onset of action. Simvastatin is a BCS Class II drug having low solubility (1.45µg/ml) and therefore low oral bioavailability (5%). SSDs of Simvastatin with two different superdisintegrants in three different drug–carrier ratios were prepared by a coevaporation method. Surface solid dispersions were characterized by differential scanning calorimetry (DSC), powder x-ray diffractometry (PXRD), scanning electron microscopy (SEM), and infrared spectroscopy (IR) and evaluated for drug content, saturation solubility, pH-dependent solubility, solubility in biorelevant media (i.e., fasted-state simulated intestinal fluid [FaSSIF] and fed-state simulated intestinal fluid [FeSSIF]), *in vitro* dissolution, and *in vivo* studies by a Triton-induced hypercholestermia model in rats. DSC studies revealed that there was no interaction between drug and carrier, whereas the PXRD study demonstrated that there was a significant decrease in crystallinity of pure drug present in surface solid dispersions, which resulted in an increased dissolution rate of Simvastatin.

Galindo-Rodriguez *et al.,* [44] formulated and optimized Repaglinide (Rg) loaded Chitosan (CN) Nanoparticles as a sustained release. Repaglinide is an oral hypoglycemic agent of the meglitinide analogue; it acts by increasing insulin secretion but binds to different beta-cell receptor sites from Sulphonylureas. Chitosan, a polycationic polymer, comprising d-glucosamine and N-acetyl-d glucosamine linked by b-(1, 4)-glycosidic bonds. Repaglinide loaded chitosan nanoparticles were prepared by solvent evaporation method in three different ratios. In this method weighed quantity of drug and polymer were dissolved in suitable organic solvent acetone and 2% acetic acid (organic phase). This solution was added drop by drop to the aqueous phase of PVA and homogenized using homogenizer at 18000 rpm followed by magnetic stirring for 2-3 h. The formed Rg-CN nanoparticles were recovered by centrifugation at 25,000 rpm for 15 min followed by washing thrice with petroleum ether and lyophilized. The prepared nanoparticles were evaluated for particle size, Scanning Electron Microscopy (SEM), Fourier Transform Infrared spectroscopy study (FT-IR), percentage yield, drug entrapment and for *in vitro* release kinetics. Among the three different ratio 1:4

ratio shown high drug loading (11.22% w/w) and encapsulation efficiencies (97.0%) and nanoparticle recovery (86.40%) with nanosize. Scanning electron microscopy exposed that nanoparticles were spherical in shape with a nearly smooth surface morphology. Particle size was analyzed by Malvern particle size analyzer and shown 48-100 nm range. FT-IR study reveals that, there was no interaction between Repaglinide and polymers. Based on the *in vitro* study, Repaglinide released from prepared formulation was slow and sustained over 15 days. Application of the in vitro drug release data to various kinetic equations indicated first order release, swelling and diffusion mechanism from Repaglinide nanoparticle.

Bathool *et al.,* [45] formulated and evaluated the sustained release nanoparticles of Atorvastatin calcium. The nanoparticles were prepared by solvent evaporation method using Chitosan as a polymer. Low oral bioavailability of Atorvastatin calcium (14%) due to an extensive high first-pass effect makes it as prime target for oral sustained drug delivery. Weighed 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. Nanoparticles were evaluated for its particle size, scanning electron microscopy (SEM), Fourier-Transform infrared spectroscopy (FTIR), percentage yield, drug entrapment and for *in vitro* release kinetics. Among the four different ratios, 1:4 ratio showed high drug loading and encapsulation efficiency. SEM studies shows that prepared nanoparticles were spherical in shape with a smooth surface. Particle size of prepared nanoparticles was found to be in the range between 142 nm to 221 nm. FTIR and DSC shows drug to polymer compatibility ruling out any interactions. *In vitro* release study showed that the drug release was sustained up to 7 days. Hence, prepared nanoparticles proved to be promising dosage form for sustained drug delivery of atorvastatin reducing dosing frequency, thus increasing the patient compliance.

Ramani *et al.,* [46] formulated and evaluated Nanoparticles of Simvastatin. Simvastatin was formulated as immediate release. Simvastatin is a poorly soluble lipid lowering agent. It's Water solubility is very low, approximately 30 μg/mL and poorly absorbed from the gastrointestinal (GI) tract. This work is an attempt to overcome the poor solubility and dissolution rate of simvastatin by using Nanosuspension technology. PVP and Tween 80 with Soybean Lecithin were used at different ratios as the surfactants. The formulations were done

by Emulsion-solvent evaporation method followed by freeze drying. The formulated nanoparticles were subjected to characterization studies like Particle size analysis, X-ray diffraction studies, Differential Scanning Colorimetry, Scanning electron microscopy and UV analysis. The dissolution test of tablets containing the nanometric drug flakes revealed that, within 30 minutes, 89.76% (w/w) of the Simvastatin in the tablet was dissolved. In comparison, the dissolution test of the conventional tablets revealed that under these testing conditions only 45.97% (w/w) Simvastatin was dissolved. This result demonstrates the significant advantage of simvastatin nanoparticles over the conventional particulate drug and the feasibility of the proposed method.

Suganeswari *et al.*, [47] prepared the nanoparticles of Hypolipidaemic drug (Atorvastatin calcium) and Antihypertensive agent (Amlodipine besylate). Nanoparticles was prepared by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic F68) and poly (D,L-lactide-co-glycolide) (PLGA) as a biodegradable polymer. Nanoparticles were characterized for size, drug loading, and *in vitro* release. Atorvastatin calcium is a second generation 3-hydroxy-3-methyl glutarylCoA reducatase inhibitor approved for clinical use as a lipid lowering agent. Atorvastatin calcium has poor oral bioavailability (12%) and serious adverse effects like rhabdomyolysis on chronic administration. Side effect of Atorvastatin was reduced 60% by combining with Amlodipine. The Amlodipine has potency to promote the activity of Atorvastatin. A biodegradable nanoparticulate approach was introduced here with a view to improving the efficacy and safety of Atorvastatin calcium. The nanoparticluate suspension of Amlodipine is to improve its absorption rate and therapeutic efficacy.

Anilkumar j Shinde *et al.*, [48] designed and evaluated Polylactic-co-glycolic acid nanoparticles containing Simvastatin. Simvastatin is a lipid lowering agent, and BCS class-II drug having low solubility and high permeability. Since Simvastatin undergoes extensive first pass extraction in the liver, the availability of the drug to the general circulation is low (< 5%). Nanoparticles were prepared by precipitation-solvent deposition method using 3^2 full factorial design. From the preliminary trials, the constraints for independent variables X1 (amount of PLGA) and X2 (amount of Pleuronic F-68) have been fixed. The prepared formulations were further evaluated for drug content, *in vitro* drug release pattern, short term stability and drug excipient interactions. The application of factorial design gave a

statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size and high entrapment efficiency. Drug: polymer ratio and concentration of stabilizer were found to influence the particle size and entrapment efficiency of Simvastatin loaded PLGA nanoparticles. *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. The release was found to follow first order release kinetics with fickian diffusion mechanism for all batches. These results indicate that simvastatin loaded PLGA nanoparticles could be effective in sustaining drug release for a prolonged period.

Vikram M. Pandya *et al.,* [49] studied the optimization of Nanosuspension of Simvastatin. Simvastatin nanosuspension was prepared by nanoprecipitation. Prepared nanosuspension was evaluated for its particle size and in vitro dissolution study and characterized by Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM). A 2^3 factorial design was employed to study the effect of independent variables, amount of PVPK-30 (X1), amount of SLS (X2) and organic to aqueous solvent ratio (X3) on dependent variables, particle size (nm) and time required to release 80% of drug (t80). The relationship between the dependent and independent variables was further elucidated using multiple liner regression analysis (MLRA). The obtained results showed that particle size (nm) and rate of dissolution has been improved when nanosuspension prepared with the higher concentration of PVPK-30 with the higher concentration of SLS and lower concentration of organic to aqueous ratio, The rate of dissolution of the optimized nanosuspension was enhanced (80% in 20min), relative to micronized suspension of Simvastatin (7.03% in 20 min), mainly due to the formation of nanosized particles. These results indicate the suitability of 2^3 factorial design for preparation of Simvastatin loaded nanosuspension significantly improved *in vitro* dissolution rate, and thus possibly enhance fast onset of therapeutic drug effect.

Adlin jino nesalin J *et al.,* [50] formulated and evaluated sustained release Nanoparticles of Flutamide. Flutamide, a substituted anilide, is a potent antiandrogenic that has been used in the treatment of prostate carcinoma having short biological half-life of 5-6 hrs; Nanoparticles of Flutamide were formulated using chitosan polymer by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analyzed for total drug content, loading efficiency, particle size and *in vitro* drug release studies. From the drug

release studies it was observed that nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.

Anilkumar J shinde *et al.,* [51] studied the effect of nanoparticle formulation on serum lipids in albino rats. The hypolipidemic effect was compared with a standard dose of Lovastatin, control and hyperlidemic control group. The rats were divided into four groups of six animals each. Test Treatment Group (TTG), Reference Treatment Group (RTG), Control Treatment Group (CTG) and Hyperlidemic Treatment Group (HTG). The treatment was given for 21 days. Each treatment group received daily standard cholesterol diet, orally in the morning throughout 21 days to induce hyperlipidemia except control treatment group. Test formulation (TF), aqueous suspensions of Lovastatin (RF) administered oral dose was 1 mg per animal once a day (equivalent to 10 mg kg–1 per day). Blood samples were collected at predetermined time intervals viz., before treatment and after 5, 10, 15 and 21 days and serum cholesterol, triglycerides and high density lipoproteins were measured. Serum levels on days 10 and 21 were compared using students paired t-test ($p < 0.001$). The results shows that plasma CH and TG levels were significantly lower (91.70±0.04 mg/dl, 145.50±0.03 mg/dl respectively) ($p < 0.001$) and HDL-CH levels were significantly higher (44.65±0.02 mg/dl, $p< 0.001$) in TTG compared to RTG. There was a significant decrease in serum cholesterol in nanoparticle formulation than standard drug.

Vikram M Pandya *et al.,* [52] formulated and evaluated Nanosuspension of Simvastatin and studied the effect of different stabilizer on the Simvastatin Nanosuspension. Simvastatin is BCS class-II drugs having low solubility and high permeability. 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 study indicate the suitability of PVPK-30 as a stabilizer in the formulation of nanosuspension.

Amir Dustgani *et al.,* [53] synthesised and characterized novel biodegradable nanoparticles of Dexamethasone sodium phosphate. Nanoparticles prepared by ionic gelation method using

Chitosan as a biodegradable polymer. Biodegradable nanoparticulate carriers, have important potential applications for administration of therapeutic molecules. Chitosan based nanoparticles have attracted a lot of attention upon their biological properties such as biodegradability, biocompatibility and bioadhesivity. Drug containing nanoparticles were prepared with different amounts of drug. The mean size and size distribution of nanoparticles were measured by dynamic laser light scattering. The mean particle size, varied in the range of 250-350 nm. Values of loading capacity and loading efficiency varied between 33.7%-72.2% and 44.5%-76.0% for prepared nanoparticles.

Riddhi Dave and Rakesh Patel [54] prepared and evaluated chitosan nanoparticles containing Doxorubicin. Nanoparticles prepared by w/o emusion method. Polymeric nanoparticles are recently more investigated for controlled and targeted drug delivery. 11 batches of nanoparticles were prepared using different concentration of oil, span 20, Chitosan and TPP. Formulations were evaluated for particle size analysis. % entrapment, Scanning Electron Microscopy, Differential Scanning Calorimetry (DSC), Infrared (IR) spectrum and *in vitro* drug release. Formulation F3 selected as an optimized formulation based on highest entrapment efficiency (53.12%) with particle size of 210nm and *in vitro* drug release.

Makarand Gambhirea, Mangesh Bhalekarb, Birendra Shrivastavaa [55] studied the solid lipid nanoparticle of Simvastatin to improve the oral bioavailability. Simvastatin is an antihyperlipidemic drug with poor oral bioavailability (<5%) due to the first pass metabolism. Simvastatin SLNs were developed using compritol 888 ATO by pre-emulsion followed by ultrasonication and characterized by photon correlation spectroscopy, DSC and XRD. 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. Simvastatin was dispersed in an amorphous state in the SLN. The results of the *in-vitro* drug release studies demonstrated significantly slow release of Simvastatin (37.08%) from SIMVA-SLN as compared to dispersion of pure drug (97.2%). The relative bioavailability of Simvastatin and Simvastatin hydroxy acid from SLN were increased by ~164% and ~207% respectively, compared with the reference Simvastatin suspension. Thus the study established that the oral bioavailability of Simvastatin could be improved by administration as SLN, presumably following digestion of constituting lipids

and co-absorption through lymphatic transport. The obtained results are indicative of SLNs as potential carriers for improving the bioavailability of poorly bioavailable drugs such as Simvastatin by minimizing first pass metabolism.

Pankaj *et al.*, [56] developed bi-layer tablets of Simvastatin using hydrophilic and or hydrophobic polymers. Simvastatin is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia. Simvastatin is a member of the statin class of pharmaceuticals, is a synthetic derivate of a fermentation product of Aspergillus terreus. The aim of present study was to prepare Simvastatin sustained release bilayer tablet by wet granulation method using hydrophilic and or hydrophobic polymers. Tablet is an important area of research in the field of drug delivery, because they the ability to deliver the wide range of drug for sustained period of time and therefore the dose and frequency of administration would be reduced hence increasing patient compliance. 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.

Athul P.V [57] formulated and characterized nanosuspension of Simvastatin (poorly soluble drug) by high pressure homogenization method to improve its dissolution characteristics and therapeutic activity. The prepared nanosuspensions were evaluated for DSC, Zeta potential analysis, SEM, solubility, *in vitro* drug release studies and *in-vivo* pharmacodynamic studies. DSC curves obtained confirms the transfer of drug crystalline form to amorphous form. Solubility studies and *in-vitro* drug release studies showed that the prepared nanosuspension has increased solubility and dissolution rate compared to pure drug. The *in-vivo* pharmacological studies showed that the nanosuspension of drug has increased anti hyperlipidemic activity compared to the pure drug. The technology was easy to scale up and requires less sophistication, the method can be extended to various poorly water soluble drugs.

Gambhire M. S. *et al.*, [58] described a Box-behnken design to optimize the formulation of Simvastatin (SIMVA) loaded solid lipid nanoparticles by pre-emulsion ultrasonication technique. The variables drug: lipid ratio, percentage of lipid phase surfactant and sonication

time were studied at three levels and arranged in a Box-behnken design, to study the influence on response variables particle size and % entrapment efficiency (%EE). From the statistical analysis of data, polynomial equations were generated. The physical characteristics of SIMVA-SLN were evaluated using particle size analyzer, differential scanning calorimetry and X-ray diffraction. The results of optimized formulation showed average particle size of 245 nm and a drug entrapment of 72.52 %. The *in-vitro* drug release study of SIMVA-SLN using modified Franz diffusion cell showed significantly low release of Simvastatin (37.08%) than dispersion of pure drug (97.2 %).

Maravajhala vidyavathi, Neelam venkata ramana [59] designed and evaluated controlled release microspheres of Simvastatin, using ethylcellulose as controlled release 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 formula was optimized by changing the polymer-drug ratio, surfactant concentration for secondary emulsion process and stirring speed of emulsification process. 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. The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicated that the release is diffusion controlled mechanism. Absence of drug-polymer interaction was also evidenced by IR & DSC thermograms. *In vivo* pharmacodynamics study of the optimized formulation proved that prolongation of drug release by presently designed microspheres.

B. Agaiah Goud *et al.*, [60] developed mucoadhesive buccal tablets of Simvastatin using mucoadhesive polymers. Simvastatin has short biological half-life (3hr), high first-pass metabolism and poor oral bioavailability (5%), hence an ideal candidate for buccal delivery system. 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 and *ex-vivo* permeation studies

through porcine buccal mucosa. Formulation FS5 and FH5 exhibited controlled drug release (8hr). Formulation FS5 selected as optimized formulation based on physicochemical parameters and *ex-vivo* permeation studies and follows zero order drug release. FTIR studies show no evidence on interaction between drug, polymers and other excipients. The results indicate that suitable bioadhesive buccal tablets for Simvastatin with desired *in-vivo* residence time and controlled release could be prepared.

BasuvanBabu *et al.,* [61] developed single-unit of oral sustained release dosage form Simvastatin (SS) have been prepared by the wet granulation method. The hydrophilic matrix was prepared with xanthan gum with additives MCC PH101. On the *in vitro* drug release was studied. The studies indicated that the drug release can be modulated by varying the concentration of the polymer and fillers. Various pharmacokinetic parameters including AUC0–t, AUC0–∞, Cmax, Tmax, T1/2, and elimination rate constant (Ke) were determined from plasma concentration of both formulations of test (Simvastatin 0.7 mg tablets) and reference (Simvastatin 1.4 mg tablets). 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 Ke were determined from plasma concentration of both Sustained and Immediate release tablets.

B.Brahmaiah *et al.,* [62] prepared and evaluated the mucoadhesive microspheres of Simvastatin. 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. Totally 15 different formulations of Simvastatin were prepared by using the above polymers. 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 hours in phosphate buffer of pH 7.0. Surface morphology (SEM analysis) and drug-polymer interaction studies (FT-IR analysis) were performed only for the ideal formulation, F10. The microspheres were smooth and elegant in appearance showed no visible cracks as confirmed by SEM and FT-IR studies indicated the lack of drug-polymer

interactions in the ideal formulation, F10. The *in vitro* release data of all microsphere formulations were plotted in various kinetic equations to understand the mechanisms and kinetics of drug release. The ideal formulation, F10 followed Higuchi kinetics and value of "n," is calculated to be 0.86 indicated that the drug release shows non-fickian diffusion.

Singla N. *et al.,* [63] compared the single dose oral bioavailability of two formulations of Simvastatin in albino rats. Plasma was analyzed for Simvastatin using a sensitive, reproducible, accurate and validated LC-MS/MS method. Pharmacokinetic parameters including AUC0-t, AUC0-∞, Cmax, Tmax, and t1/2 were determined from plasma concentration for both formulations. Self-emulsifying formulation of Simvastatin showed a significant improvement in bioavailability (1.5 fold) as compared with the conventional tablets.

P.Sandhya *et al.,* [64] formulated Hydrogel based tablets of Simvastatin using hydropropyl methyl cellulose(different grades), guar gum and carbopal-934-P studied release kinetic, to attained a near zero order release and to increaseed the bioavalability upto 95%. *In-vitro* dissolution studies were carried out using USP type 2 dissolution test apparatus. The release of drug followed a typical Higuchian pattern. Hydrogel based tablets formulated employing hydroxyl propyl methyl cellulose, guar gum and carbopal-934-P slow release of Simvastatin over period of 12 h and were found suitable for maintenance portion of oral controlled release tablets. Simvastatin release from these tablets were diffusion controlled and followed zero order kinetics after a lag time of 1h. The most successful of the study, exhibited drug release pattern very close to theoretical release profile. A decrease in release kinetics of the drug was observed.

Ana G Luque-Alcaraz *et al.,* [65] prepared and characterized Chitosan Nanoparticles containing Nobiletin. Nobiletin is a polymethoxy flavonoid with a remarkable antiproliferative effect. In order to overcome its low aqueous solubility and chemical instability, the use of nanoparticles as carriers has been proposed. This study explores the possibility of binding Nobiletin to Chitosan nanoparticles, as well as to evaluate their antiproliferative activity. The association and loading efficiencies are 69.1% and 70%,

respectively. The formation of an imine bond between Chitosan amine groups and the carbonyl group of Nobiletin, via Schiff-base, is proposed. Nobiletin-loaded Chitosan nanoparticles exhibit considerable inhibition (μg/ml) of cancerous cells, revealing their great potential for applications in cancer chemotherapy.

A.A. Kharia *et al.*, [66] formulated and evaluated nanoparticles of Acyclovir by using different hydrophilic polymers. Acyclovir was selected as a suitable drug for gastroretentive nanoparticles due to its short half-life, low bioavailability, high frequency of administration, and narrow absorption window in stomach and upper part of GIT. The nano-precipitation method was used to prepare nanoparticles so as to avoid both chlorinated solvents and surfactants to prevent their toxic effect on the body. Nanoparticles of acyclovir were prepared by using hydrophilic polymers such as bovine serum albumin, chitosan, and gelatin. The prepared formulations were then characterized for particle size, polydispersity index, zeta potential, loading efficiency, encapsulation efficiency and drug-excipient compatibility. The prepared nanoparticulate formulations of Acyclovir with different polymers in 1:1 ratio have shown particle size in the range of 250.12-743.07 nm, polydispersity index (PDI) in the range of 0.681-1.0, zeta potential in the range of -14.2 to +33.2 mV, loading efficiency in the range of 8.74-17.54%, and entrapment efficiency in the range of 55.7%-74.2%. Nanoparticulate formulation prepared with Chitosan in 1:1 ratio showed satisfactory results i.e. average particle size 312.04 nm, polydispersity index 0.681, zeta potential 33.2 mV, loading efficiency 17.54%, and entrapment efficiency 73.4%. FTIR study concluded that no major interaction occurred between the drug and polymers used in the present study.

Bhavisha Rabadiya *et al.*, [67] studied the drug polymer interaction of drug Simvastatin (SV) with different polymers like Chitosan, glycin and beta cyclodextrin(β-CD),The analytical techniques used for the purpose are Fourier Transform Infrared Spectroscopy(FTIR) and Differential Scaning Calorimeter(DSC) to characterize any drug polymer interactions and formation of inclusion complex. The complexes were prepared by simple kneading technique and were evaluated for phase solubility and aqueous solubility. The FTIR and DSC study indicate no interaction occurs between drug- polymers and revealed that no endothermic and characteristic diffraction peaks of SV was observed in the inclusion complexes. The study indicated the conversion of crystalline form of SV into the amorphous form. Aqueous

solubility profiles were markedly increased in inclusion complexes, compared with the drug alone and physical mixture this study is done so that future formulations can be prepared based on these results. Simvastatin is compatible with polymers and solubility of drug was increase by formation of inclusion complex with beta cyclodextrin(β-CD).

Parmar *et al.*, [68] formulated Strategy for Dissolution Enhancement of Simvastatin. Simvastatin is lipid lowering drug which is known as HMG CoA reductase. The objective of the study was to increase the solubility of poorly water soluble drug, namely Simvastatin, by the formation of solid dispersion and complex and also 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. For inclusion complexation method β-cyclodextrin derivative of cyclodextrin was used to prepare complex with drug. Kneading method was used for formulation. After completion of these two techniques these polymers were used for the microwave induced fusion method. All the ratio of drug and polymer were used to heat for different time interval. These samples were used for solubility measurement. 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 the microwave induced fusion method simvastatin show higher solubility with simvastatin with gelucire 44/14 after 10 mins time interval as compare to poloxamer 407 and β-cyclodextrin. Solubility of Simvastatin increased higher with gelucire 44/14 by using microwave induced fusion method as compare to other technique. By using gelucire 44/14 with Simvastatin it show 94% increase in solubility of Simvastatin as compare to pure drug in water.

AIM AND PLAN OF WORK

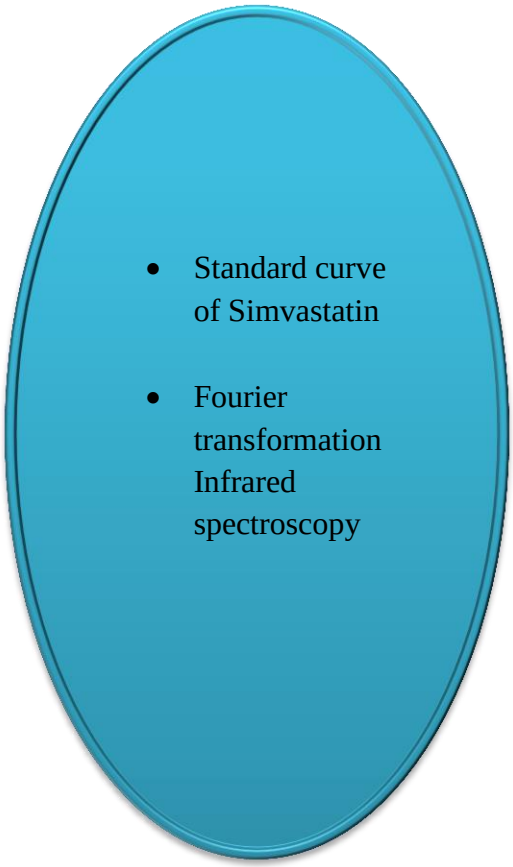
3. AIM AND PLAN OF WORK

- To formulate Simvastatin loaded Chitosan Nanoparticle
- To carry out the Characterization of Simvastatin Nanoparticles
- Identification and confirmation of purity of drug by UV Spectrophotometry.
- Pre-formulation studies
- Drug polymer compatibility studies
- Evaluation of Nanoparticles
    a) Entrapment efficiency
    b) Loading efficiency
    c) Percentage yield
    d) Drug content
    e) Solubility studies
    f) Drug particle size analysis by SEM
    g) *In-vitro* drug release studies
    h) In-vivo studies of nanoparticles as per the CPCSEA guidelines.

SIMVASTATIN LOADED CHITOSAN NANOPARTICLES

Pre- formulation studies

Simvastatin Nanoparticles



- Optimization of formulation
- PCS
- SEM
- FTIR of optimized formulation F3
- DSC
- XRD
- *In-vitro* drug release
- Kinetics of drug release
- *In-vivo* drug release

RATIONAL OF THE STUDY

4. RATIONALE OF STUDY[45, 48, 69, 70]

- The main objective of this work is to design and develop biocompatible Simvastatin nanoparticle for medical applications using natural polymer.

- Simvastatin is a better hypolipidemic drug which is used to treat various cardiovascular diseases.

- The main objective is to enhance the solubility, bioavailability and half-life of the drug. To reduce the toxicity, unwanted side effects and significant reduction of the dosage of the drug by conjugating the drug with chitosan which is used as the carrier.

- Chitosan is used as a polymer because of its advantages like biodegradability, biocompatibility, non-toxicity, non-immunogenicity, low cost, mucoadhesive and also act as stabilising agent.

- Simvastatin Nanoparticles are prepared by Nanoprecipitation method. Surfactants are not necessary which might influence the surface characteristics or cause toxic effects.

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

- No risk of cancer

DISEASE PROFILE

5. DISEASE PROFILE

5.1 ATHEROSCLEROSIS[71]

Atherosclerosis (or arteriosclerotic vascular disease) is a condition where the arteries become narrowed and hardened due to an excessive build-up of plaque around the artery wall. The disease disrupts the flow of blood around the body, posing serious cardiovascular complications.

Arteries contain *an endothelium*, a thin layer of cells that keeps the artery smooth and allows blood to flow easily. Atherosclerosis starts when the endothelium becomes damaged, allowing LDL cholesterol to accumulate in the artery wall. The body sends macrophage white blood cells to clean up the cholesterol, but sometimes the cells get stuck there at the affected site. Over time this results in plaque being built up, consisting of bad cholesterol (LDL cholesterol) and macrophage white blood cells.

The plaque clogs up the artery, disrupting the flow of blood around the body. This potentially causes blood clots that can result in life-threatening conditions such as heart attack, stroke and other cardiovascular diseases. The condition can affect the entire artery tree, but mainly affects the larger high-pressure arteries.

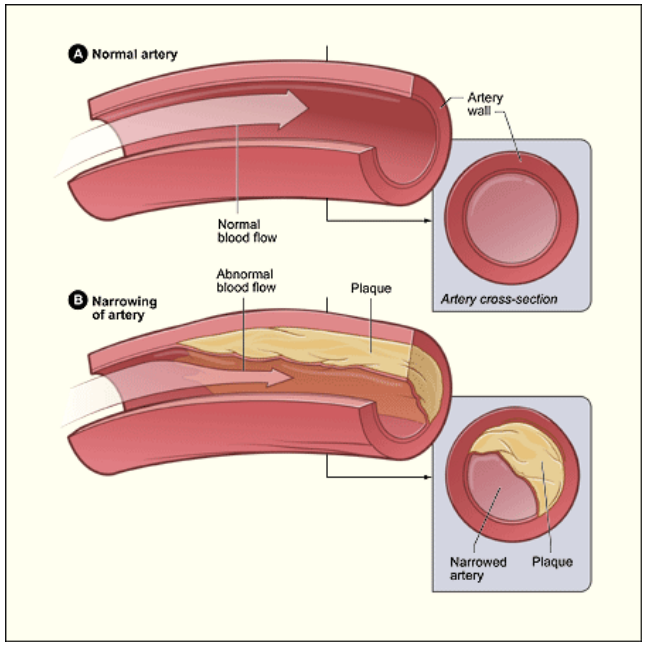


Fig.13: A represents a healthy artery. B shows an artery with plaque build-up

Signs

The first signs 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:

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, symptoms include:

- Vomiting
- Extreme anxiety
- Chest pain
- Coughing
- Feeling faint

Renal Arteries- these supply blood to the kidneys; if the blood supply becomes limited, there is a serious risk of developing chronic kidney disease, and the patient may experience:

- Loss of appetite
- Swelling of the hands and feet
- Difficulty concentrating

Peripheral arterial disease - the arteries to the limbs, usually the legs, are blocked. The most common symptom is leg pain, either in one or both legs, usually in the calves, thighs or hips.

The pain may be described as one of heaviness, cramp, or dullness in the leg muscles. Other symptoms may include:

- Hair loss on legs or feet
- Male impotence (erectile dysfunction)
- Numbness in the legs
- The colour of the skin on the legs change
- The toenails get thicker& Weakness in the legs

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 stick to an artery they secrete a molecule called netrin-1, this stops normal migration of the macrophages out of the arteries. As a result, what you have left is a 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 include:

- 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 - 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.

Air pollution - in 2007, researchers from the University of California in Los Angeles linked exposure to diesel exhaust particles in air pollution to a higher risk of bad cholesterol build-up in the arteries.

Diagnosing Atherosclerosis

Those who are at risk of developing atherosclerosis should be tested, as the symptoms don't show until cardiovascular disease develops. A diagnosis will be based on the medical history of a patient, test results and a physical exam.

Blood tests - measure sugar, fat and protein level in blood. If there are high levels of fat and sugar it can be an indicator the risk of developing the condition.

Physical exam

- The doctor will listen to the arteries using a stethoscope to see if there is an unusual "whooshing" sound reflecting turbulence of flow - called a bruit. If a bruit is heard then it can mean there is plaque obstructing blood flow.
- There may also be a very weak pulse below the area of the artery that has narrowed. Sometimes there is no detectable pulse.
- An affected limb may have abnormally low blood pressure
- There may be signs of an aneurysm (pulsating bulge) behind the patient's knee or in their abdomen
- Where blood flow is restricted, wounds may not heal properly

Ultrasound - an ultrasound scanner is able to create a picture of the inside of body using sound waves. It can check blood pressure at distinct parts of the body; changes in pressure indicate where arteries may have obstruction of blood flow.

Computed tomography (CT) scan - A CT scan uses X-ray images to create detailed pictures of the inside parts of the body. It can be used to find arteries that are hardened and narrowed.

TREATMENT FOR ATHEROSCLEROSIS

- Lifestyle changes
- Maintain a healthy weight.
- Medication or surgery.

Lifestyle Changes- 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 – Use of medications to prevent the build-up of plaque or to help prevent blood clots (antiplatelet). Other medications such as 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.

Prevention of Atherosclerosis

The best way to prevent atherosclerosis is to eliminate any risk factors you might have. The best way to do this is by living a healthy lifestyle.
Diet- To avoid saturated fats, they increase bad cholesterol level. The following foods are high in unsaturated fats and can help keep bad cholesterol levels down:

- olive oil
- avocados
- walnuts
- oily fish
- nuts
- seeds

Exercise- exercise will improve fitness level and lower blood pressure. For overweight patients, exercise can help to lose weight through activities such as walking, swimming, and cycling.

Smoking- this is one of the major risk factors for atherosclerosis, it also raises blood pressure.

Flu vaccine -Medicine in Australia said that the flu vaccination may reduce the risk of heart attack by 50% in middle aged individuals with narrowed arteries.

5.2 DYSLIPIDEMIA[72, 73]

DEFINITION

Dyslipidemia is an abnormal amount of lipids (cholesterol/fat) in the blood. In developed countries, most dyslipidemias are hyperlipidemias; that is elevation of lipids in the blood. This is often due to diet and lifestyle. Prolonged elevation of insulin levels can also lead to dyslipidemia.

TYPES OF DYSLIPIDEMIA

Table.2: Types of Dislipidemia

	Increase	Decrease
LIPID	• Hyperlipidemia -Hypercholesterolemia: due to defect of chromosome 19. -Hyperglyceridemia: glycerides -Hypertriglyceridemia triglycerides	• Hypolipidemia • Hypercholesterolemia
LIPOPROTEINS	• Hyperlipoproteinemia: lipoprotein(usually LDL) • Hyperchylomicronemia Chylomicrons	• Hypolipoproteinemia Lipoprotein • Abetalipoprotenemia: β-lioproteins • Tangier disease: HDL
BOTH	Combined hyperlipidemia: both LDL and triglycerides	

ETIOLOGY

Primary and secondary causes contribute to dyslipidemia.

PRIMARY CAUSES

Primary causes are single and multiple gene mutations that result in either overproduction or defective clearance of TG and LDL cholesterol or in underproduction or excessive clearance of HDL.

SECONDARY CAUSES

Secondary causes contribute to most cases of dyslipidemia in adults. The most important secondary causes in developed countries are a sedentary lifestyle with excessive dietary intake of saturated fat. Cholesterol and trans fat.

Diabetes is an especially significant secondary cause because patients tend to have an atherogenic combination of high TGs: high small, dense LDL fractions; and low HDL (diabetic dyslipidemia). Patients with type 2 diabetes are especially at risk.

SIGNS AND SYMPTOMS

Dyslipidemia itself usually causes no symptoms but can lead to symptomatic vascular diseases including coronary artery disease (CAD) and peripheral arterial disease.

High levels of TGs (<1000mg/dl) can cause acute pancreatitis.

High levels of LDL can cause eyelid xanthelasmas; arcuscorneae; and tendinousxanthomas at the Achilles elbow and knee tendons and over metacarpophalangeal joints.

Severe hypertriglyceridemia (>2000mg/dl) can give retinal arteries a creamy white appearance (lipemiaretinalis).

Extremely high lipid levels give a lactescent (milky) appearance to plasma.

Symptoms include Parasthesias, Dyspnea and Confusion.

DIAGNOSIS

Diagnosis is by measuring plasma levels of total cholesterol, TGs and individual lipoproteins

Diagnostic values

Table.3: Cholesterol value

Total cholesterol (U.S. and some other countries)	Total cholesterol* (Canada and most of Europe)	Cardiovascular disease
Below 200 mg/dL	Below 5.2 mmol/L	Desirable
200-239 mg/dL	5.2-6.2 mmol/L	Borderline high
240 mg/dL and above	Above 6.2 mmol/L	High

Table.4: Low density Lipoproteins value

LDL cholesterol (U.S. and some other countries)	LDL cholesterol* (Canada and most of Europe)	Cardiovascular disease
Below 70 mg/dL	Below 1.8 mmol/L	Ideal for people at very high risk of heart disease
Below 100 mg/dL	Below 2.6 mmol/L	Ideal for people at risk of heart disease
100-129 mg/dL	2.6-3.3 mmol/L	Near ideal
130-159 mg/dL	3.4-4.1 mmol/L	Borderline high
160-189 mg/dL	4.1-4.9 mmol/L	High
190 mg/dL and above	Above 4.9 mmol/L	Very high

Table.5: High Density Lipoproteins value

HDL cholesterol (U.S. and some other countries)	HDL cholesterol* (Canada and most of Europe)	Cardiovascular disease
Below 40 mg/dL (men) Below 50 mg/dL (women)	Below 1 mmol/L (men) Below 1.3 mmol/L (women)	Poor
40-49 mg/dL (men) 50-59 mg/dL (women)	1-1.3 mmol/L (men) 1.3-1.5 mmol/L (women)	Better
60 mg/dL and above	1.6 mmol/L and above	Best

Table.6: Triglyceride value

Triglycerides (U.S. and some other countries)	**Triglycerides* (Canada and most of Europe)**	**Cardiovascular disease**
Below 150 mg/dL	Below 1.7 mmol/L	Desirable
150-199 mg/dL	1.7-2.2 mmol/L	Borderline high
200-499 mg/dL	2.3-5.6 mmol/L	High
500 mg/dL and above	Above 5.6 mmol/L and above	Very high

SCREENING

A Fasting profile (TC, TG, HDL, LDL-C) should be obtained in all adults >20 years and should repeated every five years.

MANAGEMENT

The main goal of dyslipidemia management is to maintain blood cholesterol level within the normal range as possible.

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

TREATMENT

DRUGS -FOUR CLASSES OF LIPID LOWERING DRUGS ARE

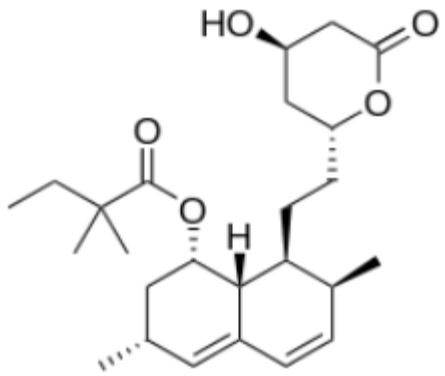
- HMG CoA reductase inhibitor(statins)
- Fibrates(gemfibrozil, clofibrate,fenofibrate)
- Niacin(nicotinic acid)
- Bile acid binding resins (colestipol.cholestyramine).

DRUG PROFILE

6. DRUG PROFILE

6.1SIMVASTATIN[74, 75]

Chemical structure



Chemical 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-dimethylbutanoate.

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

Molecular weight **:** 418.566 g/mol

Description **:** a white or almost white crystalline powder.

Melting point **:** melts between 135 and 138^{o}c

Solubility **:** very soluble in dichloromethane;

freely soluble in ethanol (95%);

practically insoluble in water.

Loss on drying **:** not more than 0.5%, determined on 1.0g by drying in an oven at 60^{o}c for 3 hrs under high vaccum.

Sulphated ash **:** not more than 0.1%

Mechanism of action

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

Pharmacokinetics

Absorption : oral absorption of Simvastatin is found to be 42.5%

Bioavailability : 5%

Half-life : 3 hours

Plasma protein binding : ~95% bound to plasma proteins.

Metabolism : extensively hepatic; undergoes metabolism by CYP3A4.

Excretion : renal 13%; fecal 60%

Therapeutic indications

- The primary uses of Simvastatin are for the treatment of dyslipidemia.
- It is also used for the prevention of cardiovascular disease.
- Used in homozygous familial hypercholesterolemia.

Route/dosage

For hyperlipidemia

Adult dose - 10 to 20 mg orally once in the evening

Children less than 10 years - 5 mg once daily in evening.

Cardiovascular risk reduction - 10 to 20 mg orally once in the evening.

Contra indications

Simvastatin is contraindicated in pregnancy, breast feeding and liver diseases. It is also contra indicated with amlodipine.

Adverse effects

Abdominal pain, diarrhoea, indigestion and a general feeling of weakness.

Drug interactions

CYP3A4 inhibitors like Itraconazole, Ketoconazole, Erythromycin, Amiodaron , etc. decreases the metabolism of simvastatin.

Grape fruit contains furanocoumarin which slows down the metabolism of simvastatin.

Combination with other drugs

Simvastatin+Ezetimibe

Simvastatin+Niacin.

EXCIPIENT PROFILE

7. EXCIPIENT PROFILE

7.1CHITOSAN[76, 77]

Nonproprietary names

- BP: Chitosan hydrochloride
- PhEur: Chitosani hydrochloridum

Synonyms

2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; deacetylated chitin; deacetylchitin; b-1,4-poly-D-glucosamine; poly-D-glucosamine; poly-(1,4-b-D-glucopyranosamine).

Chemical name and CAS registry number

Poly-b-(1, 4)-2-Amino-2-deoxy-D-glucose (9012-76-4)

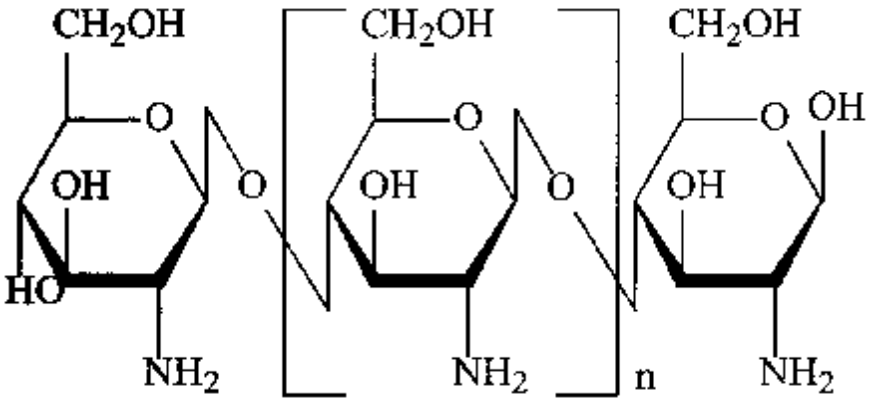
Empirical formula and molecular weight

Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerization and it is therefore not easily defined in terms of its exact chemical composition.

A clear nomenclature with respect to the different degrees of N-deacetylation between chitin and chitosan has not been defined and as such chitosan is not one chemical entity but varies in composition depending on the manufacturer. In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80–85%.

Chitosan is commercially available in several types and grades that vary in molecular weight by 10000–1000000, and vary in degree of deacetylation and viscosity.

Structural Formula



Structure of chitosan

Functional category

Coating agent; disintegrant; film forming agent; mucoadhesive; tablet binder; viscosity increasing agent.

Description

Chitosan occurs as odorless, white or creamy white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look ‘cottonlike’.

Typical properties

Chitosan is a cationic polyamine with a high charge density at pH <6.5; and so adheres to negatively charged surfaces and chelates metal ions. It is a linear polyelectrolyte with reactive hydroxyl and amino groups (available for chemical reaction and salt formation). The properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. The presence of a number of amino groups allows chitosan to react chemically with anionic systems, which results in alteration of physicochemical characteristics of such combinations. The nitrogen in chitosan is mostly in the form of primary aliphatic amino groups. Chitosan therefore undergoes reactions typical of amines: for example, N-acylation and Schiff reactions. Almost all functional properties of chitosan depend on the chain length, charge density, and charge distribution. Numerous studies have demonstrated that the salt form, molecular weight, and degree of deacetylation as well as pH at which the chitosan is used all influence how this polymer is utilized in pharmaceutical applications.

Table.7: Properties of Chitosan

PARAMETERS	DESCRIPTION
Appearance (powder or flake)	White or yellow
Particle size	Less than 30 μm
Viscosity (1% solution/ 1% acid)	Less than 5 cps
Density	Between 1.35 to 1.40 g/cm^3
Molecular weight	50,000 to 2,00,000 Da.
pH	6.5 to 7.5
Moisture content	More than 10%
Ash value	More than 2%
Mater insoluble in water	0.5%
Degree of acetylation	66% to 99.8%
Heavy metal (Pb)	Less than 10 ppm
Heavy metal (As)	Less than 10 ppm
Protein content	Less than 0.3%
Loss on drying	Less than 10%
Glass transition temperature	203^oC

Moisture content

Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Solubility

Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulphuric acids).

Viscosity (dynamic)

A wide range of viscosity types is commercially available. Owing to its high molecular weight and linear, unbranched structure, chitosan is an excellent viscosity enhancing agent in an acidic environment. It acts as a pseudo plastic material, exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solutions increases with increasing chitosan concentration, decreasing temperature, and increasing degree of deacetylation.

Stability and Storage Conditions

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place.Chitosan should be stored at a temperature of 2–8^{o}C.

Preparation of Chitosan (CS) from Raw Materials

CS is not a single chemical entity, but varies in composition depending on the source and method of preparation defined as sufficiently deacetylation of chitin to form a soluble amine salts. The degree of deacetylation must be 80 to 85% or higher or the acetyl content must be less than 4-4.5% to form the soluble product. CS is manufactured commercially by a chemical method. Firstly the sources such as crab or shrimp shells are washed and grinded in to powdered form and then it is deproteinized by treatment with an aqueous 3-5% solution of sodium hydroxide. After that it is neutralized and demineralized at a room temperature by treating it with aqueous 3-5% of hydrochloric solution to form a white or slightly pink precipitate of chitin. Then chitin is deacetylated by treatment with an aqueous 40-45% of sodium hydroxide solution and the precipitate is then washed with water. The insoluble part is removed by dissolving in an aqueous 2% acetic acids solution. The supernatant solution is then neutralized with an aqueous sodium hydroxide solution to obtain a purified CS

Incompatibilities

- Chitosan is incompatible with strong oxidizing agents.

Safety

Chitosan is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. It is also used in cosmetics. Chitosan is generally regarded as a nontoxic and nonirritant material. It is biocompatible with both healthy and infected skin.Chitosan has been shown to be biodegradable.

LD50 (mouse, oral) >16 g /kg.

Applications in Pharmaceutical Formulation:

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications

- Component of mucoadhesive dosage forms
- Rapid release dosage forms
- Improved peptide delivery
- Colonic drug delivery systems
- Use for drug & gene delivery

Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets and coating for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spray-drying, coacervation, direct compression and conventional granulation process.

MATERIALS AND METHODS

8. MATERIALS & METHODS

List of chemicals used

Table.8: List of chemicals

S.No	Name of the material	Manufacturer	Use in formulation
1.	Simvastatin	Orchid chemicals, Chennai.	Active ingredient
2.	Chitosan	Lab Chemicals, Chennai.	Polymer
3.	Acetic acid	Alan Medicals & Laboratory products, Chennai.	Solvent
4.	Acetone	RFCL Limited, New Delhi.	Solvent
5.	Cholesterol	Micro Fine Chemicals, Chennai.	Fatty diet
6.	Sodium Cholate	Kemwell pharmaceuticals, Bangalore.	Fatty diet
7.	Coconut oil	Home made.	Fatty diet
8.	Carboxy methyl cellulose	Chenchems, Chennai.	Suspending agent
9.	Acetic anhydride	Medical supplies, England.	Reagent
10.	Sulphuric Acid	Paxmy speciality chemicals, Chennai.	Reagent
11.	Sodium tungstate	LC Industrials, Mumbai.	Reagent
12.	Phosphoric acid	Chenchems, Chennai.	Reagent
13.	Calcium chloride	Indian Research Products, Chennai.	Reagent
14.	Ether	TKM pharma, Hydrabad.	General anaesthesia
15.	Potassium dihydrogen Phosphate	Merck specialities Pvt.Ltd, Mumbai.	Buffer
16.	Sodium hydroxide	Indian Research Products, Chennai.	Buffer
17.	Methanol	Supra chemicals, Chennai.	Solvent
18.	Glycerin	Microfine chemicals, Chennai.	Humectant
19.	Potassium bromide	Merck, Germany.	Medium

List of instruments/ equipment's used

Table.9: List of instruments

S.No	Equipments/Instruments	Manufacturer
1.	Electronic weighing balance	Shimadzu, Japan.
2.	Magnetic stirrer	Remi.
3.	Hot Air Oven	Industrial heaters, Chennai.
4.	High speed homogenizer	Remi, Vasai.
5.	Cooling centrifuge	Remi, Vasai.
6.	Lyophilizer	Remi, Vasai.
7.	UV- Visible Spectrophotometer	Shimadzu, Japan.
8.	Fourier Transform Infrared Spectrophotometer	Nicolet, India.
9.	Olympus Binocular Microscope	Remi.
10.	pH meter	Symchrony. India.
11.	Ultrasonic cell crusher	Lark.

FORMULATION DEVELOPMENT

9. FORMULATION DEVELOPMENT

PREFORMULATION STUDIES[78]

The pre-formulation studies are the first step in the rational development of any formulation. It can be defined as the "investigation of physical and chemical properties of the drug substance alone and combined with excipients". These studies focus on those physicochemical properties of new compound that could affect drug performance and development of an efficacious formulation. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced. The type of information needed will depend on the dosage form to be developed.

Thus the goals of the final study are,

1. To establish physical characteristics
2. To establish its compatibility with the excipient
3. To determine kinetic rate profile

Calibration Curve of Simvastatin in 6.8pH Phosphate Buffer[79]

Preparation of 6.8pH Phosphate buffer

- 0.2M solution of potassium dihydrogen phosphate was prepared by dissolving 27.218gm of substance in 1000ml of distilled water.
- 0.2M solution of sodium hydroxide solution was prepared by dissolving 8gm of substance in 1000ml of distilled water.
- 50ml of above prepared potassium dihydrogen phosphate solution & 22.4ml of sodium hydroxide solution were mixed together and made upto 2000ml and the pH was adjusted to 6.8.

DETERMINATION OF λ max[46]

A stock solution of 1 mg/ml of Simvastatin was prepared by dissolving 100 mg of drug in small quantity of ethanol and sonicated for few minutes. The solution was diluted

with 100 ml of phosphate buffer (pH 6.8) and serially diluted to get solution in the range of 20µg/ml. The λ max of the solution was found out by scanning from 200 - 400 nm. The λmax of Simvastatin was used for further quantitative analysis.

CALIBRATION CURVE

A stock solution of 1 mg/ml of Simvastatin was prepared by dissolving 100 mg of drug in small quantity of methanol and sonicated for few minutes. It was diluted to 100ml with phosphate buffer (pH 6.8). The stock solution was serially diluted to get solutions in the range of 2-10µg/ml. The absorbance of the different diluted solutions was measured in a UV-Visible spectrophotometer at 238 nm. A calibration curve was plotted taking concentration of solution in X axis and absorbance in Y axis and correlation coefficient 'r' was calculated.

Polymer compatibility studies[48]

The proper design and formulation of a dosage form requires consideration for physical, chemical and biological characteristics of all drug substances and excipients used in fabricating the final product. The successful formulation of a stable and effective formulation depends on the selection of excipients. The drug and the excipient must be compatible with one another to produce a stable product efficacious and easy to administer.

Fourier Transformation Infrared Spectroscopy:

Compatibility study was studied by recording the sample using Perkin Elmer Fourier transformation Infrared spectroscope (FT-IR) combined to PC (with spectrum 2000 analysis software) in the range of 4000 cm^{-1} to 400 cm^{-1}. Potassium Bromide Press Pellet was prepared by applying pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in light path and the spectra were analysed.

Composition for FT-IR Study -Table.10

S.NO	INGREDIENTS
1.	Chitosan
2.	Simvastatin
3.	Chitosan and Simvastatin admixture

Preparation of chitosan nanoparticles[66]

Nanoparticles are prepared by Nano precipitation -solvent deposition method using chitosan as a coating material and Simvastatin as a core material. Drug and polymer in different ratios were weighed and dissolved in a suitable organic solvents, Acetone & 1.5% Acetic acid respectively. Both solutions were mixed and added drop wise into water, mixed at 3000 rpm for 2 hrs forming a milky colloidal suspension and homogenised at 25000 rpm. The resultant nanoparticle suspension was recovered by centrifugation (REMI cooling centrifuge) at 12000 rpm for 30 min and lyophilized.

Table.11: Formulation Table

S.No	Formulation	Amount of drug (mg)	Concentration of Chitosan (%)	Acetone (ml)	Acetic acid (ml)	Dis.water (ml)
1.	F1	50	0.1	5	50	60
2.	F2	50	0.2	5	50	60
3.	F3	50	0.3	5	50	60
4.	F4	50	0.4	5	50	60

Characterization:

Percentage yield[80]

The nanoparticle yield was calculated according to the equation given below.

$$\text{Process Yield (\%)} = \frac{\text{Mass of nanoparticles}}{\text{Total mass of drug + polymer}} \times 100$$

Loading efficiency & Entrapment efficiency[66]

The Nanosuspension with known amount of drug was centrifuged at 5000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 50ml of phosphate buffer solution pH 6.8 and the absorbance was measured using UV spectrophotometer at 238 nm using phosphate buffer solution pH 6.8 as blank. The amount of

drug unentrapped was calculated. The percentage of entrapment and loading efficiency was determined according to the equation given below.

$$\text{Loading efficiency (\%, w/w)} = \frac{\text{Total amount of drug - Amount of unbound drug}}{\text{Nanoparticles weight}} \times 100$$

$$\text{Drug entrapment (\%, w/w)} = \frac{\text{Mass of the total drug} - \text{Mass of free drug}}{\text{Mass of total drug}} \times 100$$

DETERMINATION OF DRUG CONTENT[46]

Equivalent to 1mg of the prepared formulations were weighed and dissolved in minimum quantity of Ethanol and made up to 50ml with phosphate buffer (6.8pH). The absorbance of the solutions was measured at 238nm. The drug content was calculated.

Optimization of formulation

Photon Correlation Spectroscopy (PCS) [45]

The particle size of the resulting nanoparticles was determined by PCS. A BI-200SM Goniometer Version 2.0 (Marlvern Instruments, Germany). The particle size was expressed by the effective diameter and the width of size distribution was characterized by polydispersity index. The particle size, their range and degree of distribution were studied for the best formulation.

Zeta Potential Measurements[81, 82]

Zeta potential measurements are generally performed to determine the stability of the nanoparticles.

Three of the fundamental states of matter are solids, liquids and gases. If one of these states is finely dispersed in another then it is known as “colloidal system”. In certain circumstances, the particles in a dispersion may adhere to one another and form aggregates of successively increasing in size, which may settle out under the influence of gravity.

Zeta potential is a physical property which is exhibited by any particle in a colloid, suspension or emulsion. It can be used to optimize the formulations. Knowledge of the zeta potential can reduce the number of trials in formulations. It is also useful in predicting long-term stability.

The particles in a colloidal suspension usually carry an electrical charge. The charge is more often negative than positive. Sometimes the surface of the particles which is containing chemical groups can ionize to produce a charged surface. Sometimes the surface itself preferentially adsorbs ions of one sign of charge in preference to charges of the opposite sign. In other cases there may be deliberately added chemical compounds that preferentially adsorb on the particle surface to generate the charge.

However it may happen, the amount of charge on the particle surface is an important particle characteristic because it determines many of the properties of the colloids.

a) Zeta Potential Range

The zeta potential is the overall charge a particle acquires in a specific medium.

- The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system.
- If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability.
- If the particles have low zeta potential values then there is no force to prevent the particles coming together and there is dispersion instability.
- A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30mV.
- Particles with zeta potentials more positive than +30mV are normally considered stable.
- Particles with zeta potentials more negative than -30mV are normally considered stable.

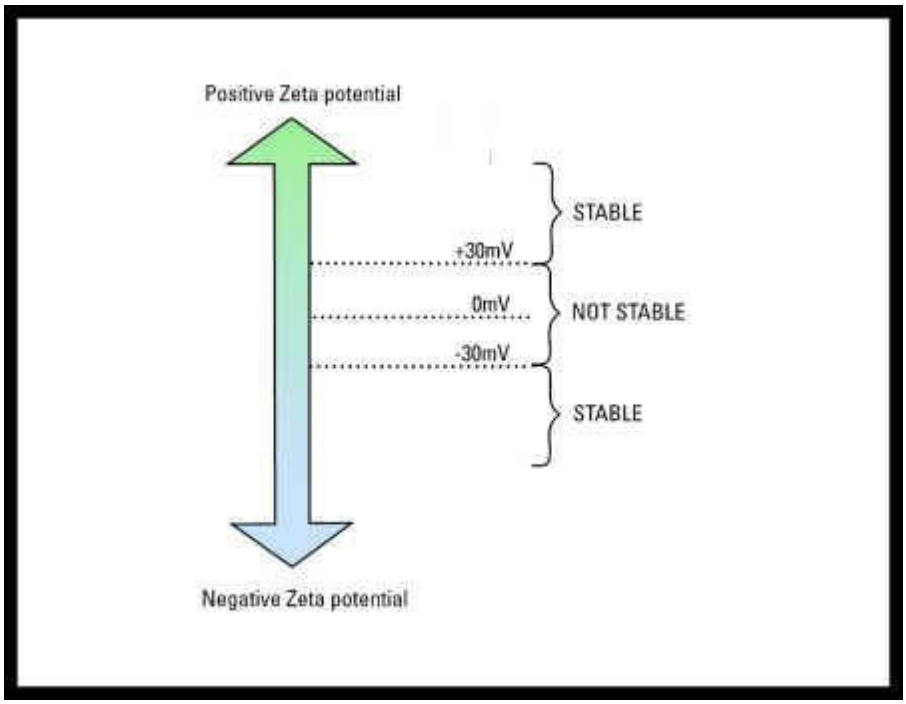


Fig.14: Zeta potential

Procedure:

Zeta potential is done using water as a dispersant which has refractive index 1.330, viscosity 0.8872 and dielectric constant 78.5 at 25˚C.

Scanning electron microscopic (SEM)

SEM photographs were taken with a scanning electron microscope [Model Vega3-Tescan, USA] at the required magnification at room temperature. The Nanoparticles, pure Simvastatin were deposited on a glass disc applied on a metallic stub separatly and evaporated under a vacuum overnight. Before the SEM analysis, the samples were metallized under an argon atmosphere with a 10-nm gold palladium (EMITECH- K550 Sputter Coater, Houston, TX).

DIFFERENTIAL SCANNING COLORIMETRY (DSC)

Differential scanning colorimetry (DSC) was conducted on Diamond DSC Calorimeter at Anna University. The samples were equilibrated at 20° C for half hour and then heated to 220° C at 10° C/ml in a N2 atmosphere.

FTIR of formulation F3:

The compatibility study was carried out for the best formulation using Perkin Elmer FTIR at wavelength range of 4000 to 400 cm^{-1} to confirm any interaction between the drug and polymer.

X-RAY DIFFRACTION STUDY

X-ray diffraction analysis was employed to detect the crystallinity of the pure drug and the formulations, which was conducted using a XRD-6000 diffractometer (Shimadzu, Japan) at Anna University. The powder was placed in a glass sample holder. CuK radiation was generated at 30mA and 40 kV. Samples were scanned from 5° to 50° with a step size of 0.02° and the scan speed was 3° min^{-1}

In-vitro drug release characteristics[83]

The *in-vitro* drug diffusion from the formulation was studied using cellophane membrane. Phosphate buffer pH 6.8 was used as a dissolution medium solution. Cellophane membrane was previously soaked in the mixture of glycerol & water (1:4 ratio) for 20minutes and the cellophane membrane was tied to one end of a specially designed glass cylinder (open at both ends). 10 ml of formulation was accurately placed into this assembly. The cylinder was suspended in 200 ml of dissolution medium maintained at 37 ± 5ºC. The dissolution medium was stirred at 100 rpm using magnetic stirrer. 10 ml samples were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The aliquots were analyzed by UV-Vis Spectrophotometer at 238 nm. The cumulative % release of the formulations was calculated.

PHARMACOKINETICS

In-vitro release kinetics (theoretical calculation from % Cumulative drug release).

To study the release kinetics, data obtained from *in-vitro* release were fitted in various kinetic models.

a) **Zero order reaction:**

A graph was plotted with % drug released vs. time.

$$C=K_0t$$

Where K_0 - Zero order rate constant

t - time

C- % drug released

b) **First order reaction:**

A graph was plotted with log cumulative % drug remaining vs. time.

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

Where C_0 - Initial concentration of drug.

K-First order constant, t - time.

c) **Higuchi kinetics:**

A graph was plotted with cumulative % drug released vs. square root of time

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

Where K - constant reflecting design variable of system. (Differential rate constant)

t - time.

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 particular dosage form is considered to follow Higuchi kinetics of drug release.

d) **Hixson Crowell erosion equation:**

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

$$Q_0^{1/3} - Q_t^{1/3} = KHC \times t$$

Where Q_t - Amount of drug released in time t.

Q_0 - Initial amount of drug

KHC - Rate constant for Hixson Crowell equation.

e) **Korsmeyer –Peppas equation:**

To evaluate the mechanism of drug release, the drug release was fitted in Peppas equation with log cumulative of drug released vs. time.

$$Mt/M\alpha = Ktn$$

$$\text{Log } Mt/M\alpha = \log K + n \log t$$

Where $Mt/M\alpha$ –fraction of drug released at time t

K - kinetic constant (incorporating structural and geometric characteristics of preparation)

n- diffusional exponent indicative of the mechanism drug release.

If n value is 0.5 or less, the release mechanism follows “Fickian diffusion”. 0.5 to 1 it follows a non-fickian model (anomalous transport).The drug release follows zero-order drug release and case II transport if the n value is 1. For the values of n higher than 1, the mechanism of drug release is regarded as super case II transport. This model is used to analyse the release of pharmaceutical polymeric dosage forms when the release mechanism is not known or more than one type of release phenomenon is involved. The n value could be obtained from slope of the plot of log cumulative % of drug released vs. log time.

Stability studies

Stability of Simvastatin nanoparticles was carried out at room temperature, refrigeration and accelerated condition at 40^0C/75% RH for a period of 3 months. Then the samples were analyzed spectrophotometrically.

***In-vivo* Study**[51]

Selection of animal species

The albino rats (Wistar strain) of either sex having weight 150 to 250 g were used for testing antilipidemic activity.

Experimental animals

Animals had free access to food and water ad libitum. The effect of optimized formulation (test formulation) on plasma lipid profiles was determined and compared with reference formulation (aqueous suspension containing Simvastatin equivalent to 10 mg/ kg and carboxy methyl cellulose 0.25% w/v as a suspending agent) in healthy albino rats. The animals were randomly divided into 4 treatment groups of six animals each, viz., Control Treatment Group (CTG), Hyperlipidemic Treatment Group (HTG), Reference Treatment Group (RTG) and Test Treatment Group (TTG). The treatment was given for 21 days. Each treatment group received daily standard cholesterol diet (2% coconut oil, 2% cholesterol and 1% sodium cholate) orally in the morning throughout 21 days to induce hyperlipidemia except control treatment group. High cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% ,and coconut oil 2% with standard powdered animal food. The diet was placed in the cage carefully for 21 days for hyperlipidemic control group, reference treatment group and test treatment groups.

Collection of Blood samples

Blood samples were collected under light ether anaesthesia by retro-orbital puncture at predetermined time intervals, viz., before treatment, and after 0, 5, 10, 15, 21 days in centrifuge tube. Plasma was separated by centrifugation at 3000 rpm for 25 min and stored frozen until further use.

Biochemical analysis

The lipid profiles -Total Cholesterol (CH), triglycerides (TG), low density lipoproteins (LDL-C),very low density lipoproteins(VLDL) and high density lipoproteins (HDL-C) levels) were estimated to confirm the induction of hyperlipidemia. LDL-C and AI, in plasma was calculated as per Friedewald estimation. Fixed volumes of sample and standard were mixed with the working reagent and incubated at 37°C for 10 min. The absorbance of the developed colour was read at 505 nm for CH and at 546 nm for TG.

ESTIMATION OF CHOLESTEROL[84]

A single stable Liebermann-Burchard reagent was prepared and used for the rapid and accurate determination of total cholesterol in serum.

Preparation of Libermann-Burchard reagent

In the preparation of the Liebermann-Burchard (LB) reagent, the following reagents are necessary: (1) glacial acetic acid, AR; (2) acetic anhydride, AR; (3) sulfuric acid, AR. It is very important that all glassware for the preparation of this reagent be chemically clean and dry.

Acetic anhydride and concentrated sulfuric acid were cooled in ice water. 24 ml of cold Acetic anhydride and 22 ml of Glacial Acetic acid (room temperature) were added to an amber coloured glass bottle. It was mixed by inversion, 4 ml of cold concentrated sulfuric acid, the reagent was added and mixed.

It is stable for more than 6 months when stored at 4^0c in the dark. When stored at room temperature, the reagent shows signs (darkening) of deterioration after 1-2 weeks.

Standard Curve

A 200 mg/100 ml cholesterol standard was prepared by dissolving 100 mg cholesterol in 50 ml glacial acetic acid and stored in an amber glass bottle.

Procedure

- The test tubes were labelled as 'C', 'H', 'R', 'T', 'S' and 'B'. 6ml of LB reagent was pipetted.
- To the 'C', 'H', 'R' and 'T' test tubes, 0.2ml of unknown serum from respective groups of animals was added. To the 'S' test tube, 0.2ml of cholesterol standard was added and mixed with the aid of vortex mixer.
- All the test tubes were incubated at 37^0c for 18 minutes.
- The tubes were wiped and the absorbance was measured at 505 nm at regular timed intervals over the next 10 minutes, using LB reagent as blank.
- Using the standard photometric formula, the concentration of the unknown is calculated.

$$C_u = C_s \, X \, (A_u/A_s)$$

Where, C_u, C_s - Concentration of unknown and standard respectively.

A_u, A_s - Absorbance of unknown and standard respectively.

ESTIMATION OF TRIGLYCERIDES USING COLORIMETRY[85]

Sample preparation

- The blood was collected without using an anticoagulant
- It was allowed to clot for 30 minutes at 25^oC
- The blood was centrifuged at 2000rpm for 15minutes at 4^0C. The top yellow serum layer was pipetted out without disturbing the white buffy layer. The serum was stored on ice and assayed.
- Calculate the values of triglyceride samples using the equation obtained from the linear regression of the standard curve by substituting the corrected absorbance values for each sample into the equation

$$\text{Triglycerides (mg/dl)} = \frac{\text{Corrected absorbance} - \text{(y-intercept)}}{\text{Slope}}$$

ESTIMATION OF HDL BY PTA (PHOSPHO TUNGSTIC ACID) METHOD[86]

PROCEDURE

- The tube was labelled for reagent blank, standard and unknown sample (test).
- 1.0 ml of reagent was added to each tube and brought to assay temperature (25°, 30° or 37°C).
- 0.05ml of water was added to the reagent blank. 0.05ml of standard and sample was added to the appropriate tube, mixed and incubated at 37°C for 6 minutes
- The absorbance was measured.
- The concentration of unknown were calculated using the following formula

$$C_u = C_s \text{ X } (A_u/A_s)$$

Atherogenic index, VLDL and VLDL was calculated using the following formula

ATHEROGENIC INDEX[87]

$$AI = \log (TG/HDL)$$

LOW DENSITY LIPOPROTEINS

$$LDL = TC- HDL-(TG/5)$$

VERY LOW DENSITY LIPOPROTEINS

$$VLDL = TG/5$$

RESULTS AND DISCUSSION

10. RESULTS AND DISCUSSION

Pre-formulation Studies:

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

UV-visible spectroscopy and FTIR spectroscopy gives the possible information about the interaction between the drug and polymer.

Values for standard curve of Simvastatin at 238nm.

Table.12: Absorbance of Simvastatin

S.No	Concentration µg/ml	Absorbance
1.	2	0.131
2.	4	0.264
3.	6	0.393
4.	8	0.524
5.	10	0.645

Inference:

The ultraviolet spectrophotometric method was used to analyze Simvastatin at wavelength of 238nm. Absorbance of the Simvastatin shown in Table.12. The standard plot was prepared in phosphate buffer 6.8. It showed linearity at concentration range of 2 to 10 µg/ml shown in Fig.15.

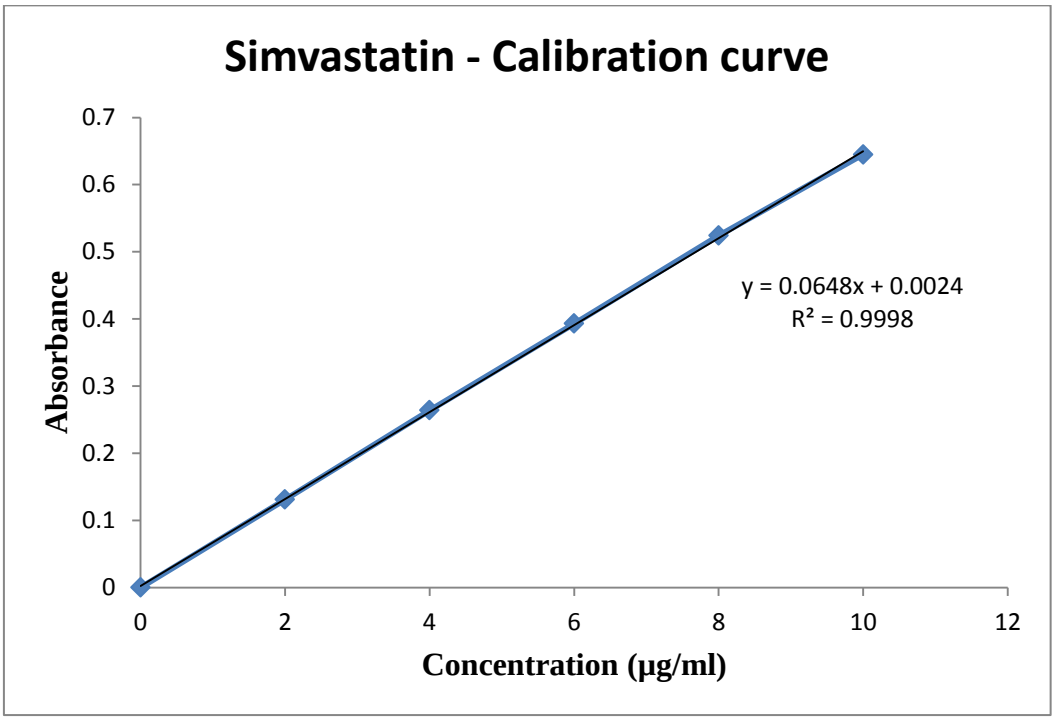


Fig.15: Simvastatin Calibration curve

Drug – polymer compatibility studies:

The physical state of Simvastatin, Chitosan and physical admixture of the Chitosan & Simvastatin were subjected to FTIR spectroscopy.

FTIR studies:

Comparison of IR peaks of polymer and drug admixtures.

Table.13: FTIR peaks

S.No	Samples	Wave number(cm^{-1})
1.	Chitosan	3394, 2923, 2885, 1650, 1589, 1419, 1380, 1319, 1072.
2.	Simvastatin	3548, 3417,2962,2877,1836, 1704, 1458, 1388, 1265, 1064.
3.	Chitosan & Simvastatin	3749, 3548, 3417, 2954,2877,1828,1704, 1458,1388,1265,1064.

FTIR of Simvastatin

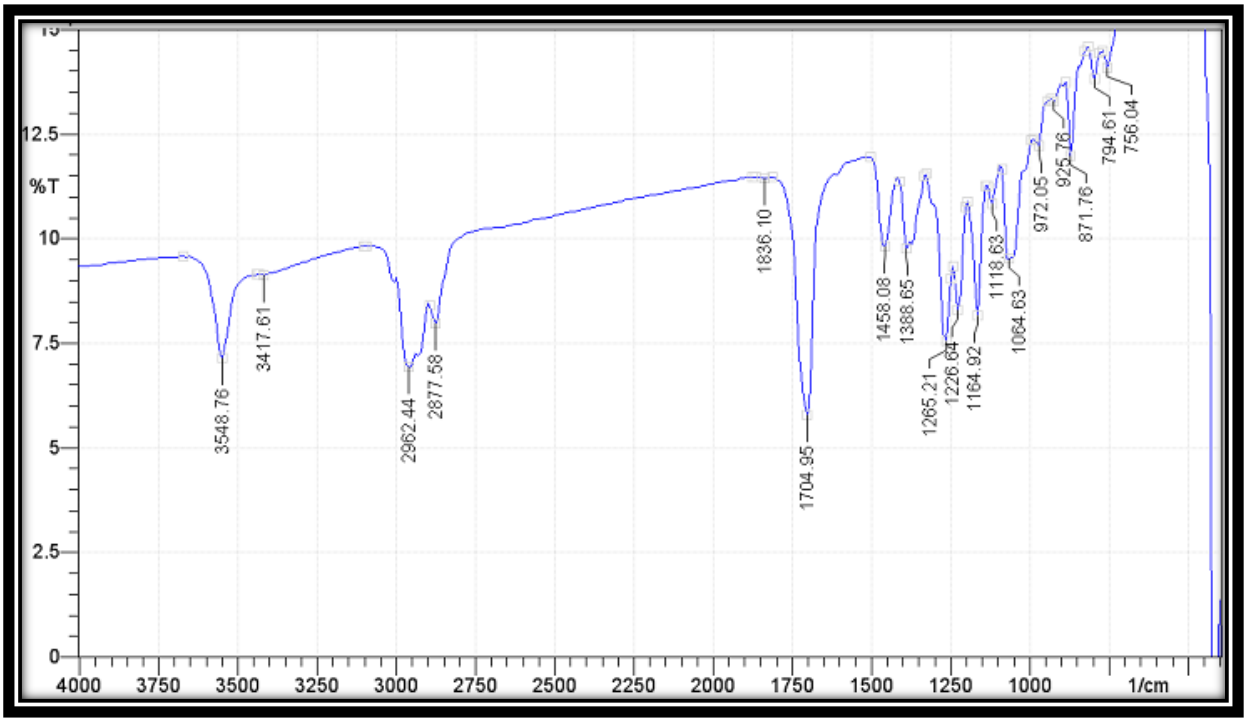
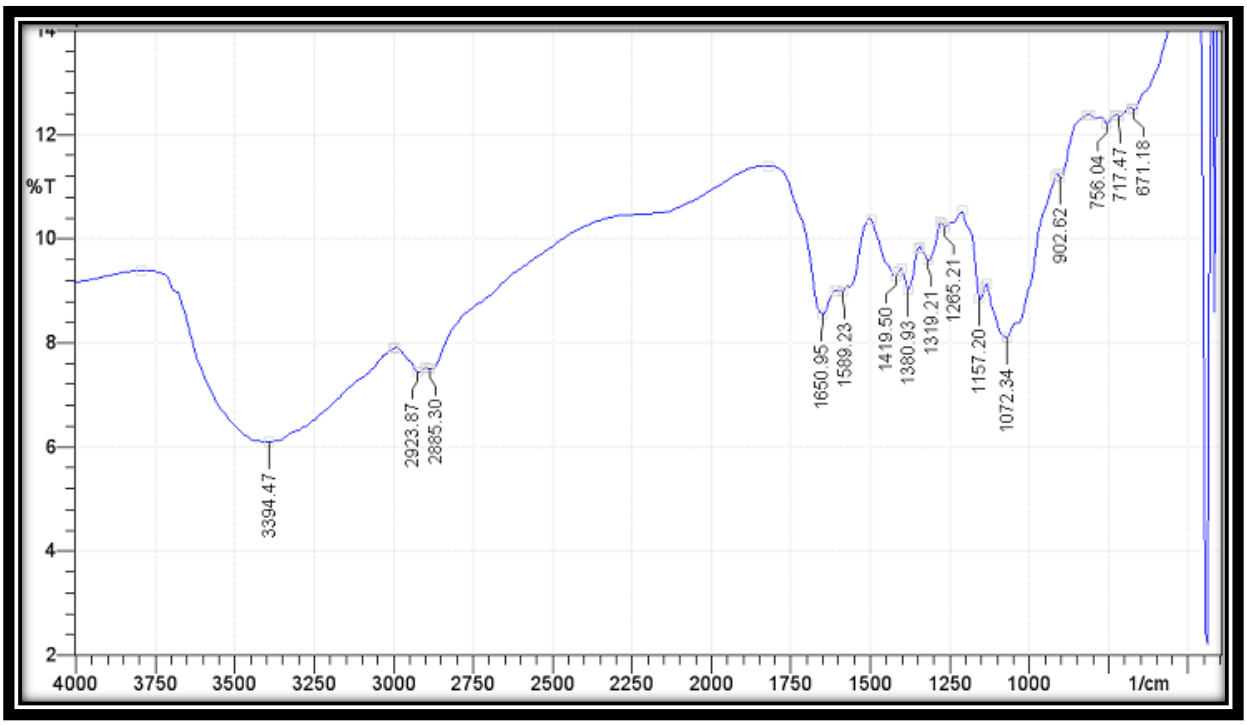


Fig.16: FTIR of Simvastatin

FTIR of Chitosan- Fig.17



FTIR data of Simvastatin and Chitosan

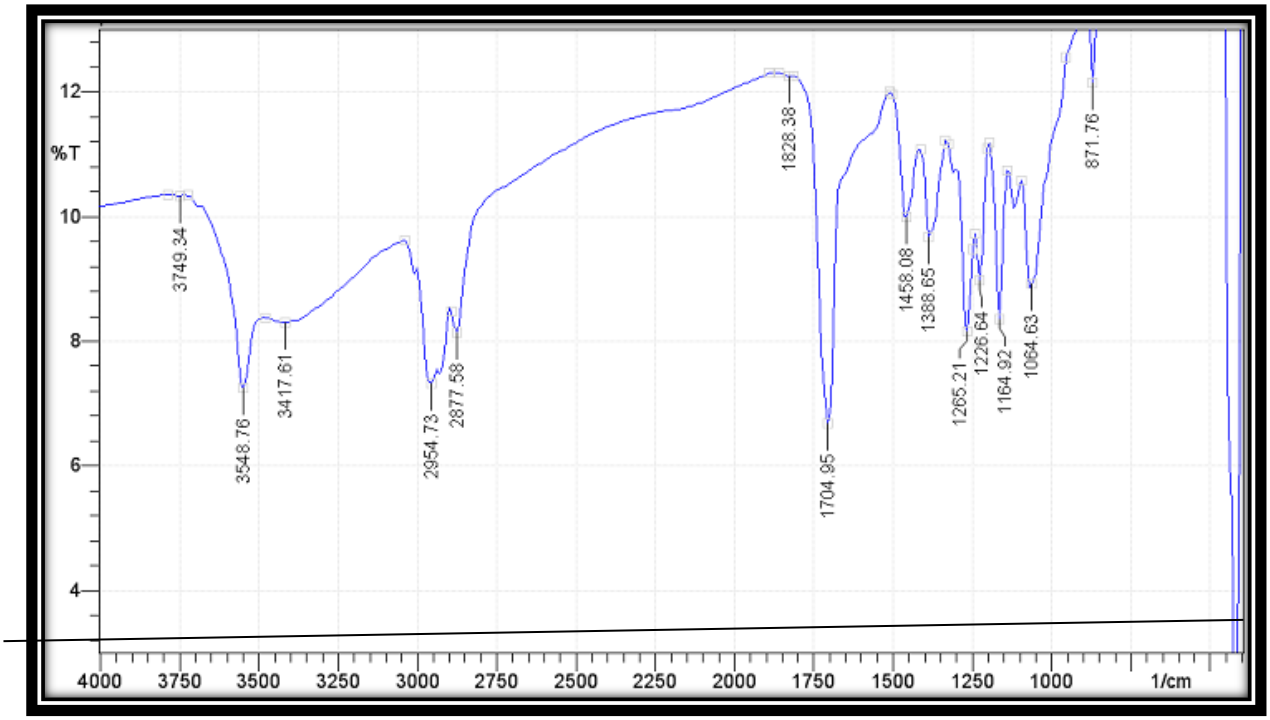


Fig.18: FTIR of Simvastatin and Chitosan

Inference

The IR spectra are shown in the figures16, 17 and 18. No change in peaks of admixture compared with drug indicates the absence of any interaction.

FTIR data of simvastatin- Table.14

S.No	Functional group	Standard wave number	Peak observed in Simvastatin API ($cm^{-1)}$
1.	Free O-H stretch	3546	3548
2.	Methyl C-H symmetric stretch; Methylene C-H asymmetric stretch	2924	2962
3.	Ester C =O stretch	1697	1704
4.	Methylene C-H symmetric bend, Methyl C-H symmetric bend	1461	1458
5.	Lactone-C-O-C bend	1268	1265
6.	Ester-C-O-C-bend	1164	1164
7.	Secondary alcohol C-O stretch	1072	1064
8.	N-H stretching	1568	1836

FT-IR data of chitosan- Table.15

S.No.	Functional group	Peak observed in chitosan (cm-1)
1.	N-H symmetric stretching vibration	3450
2.	OH stretching	3394
3.	C-H stretching vibration	2923
4.	NH bending (amide II) (NH_2)	1560,1639 ,1319
5.	C=0 stretching (amide I) 0=C-NHR.	1647
6.	CH_2 bending	2927, 2885, 1419, 1319, 1265
7.	CH_3 wagging	1380 and 1319
8.	>CO-CH_3 stretching vibration	1072
9.	Confirmed a saccharide structure	1265, 1157

FTIR data of simvastatin and Chitosan- Table.16

Interpretation	Wave number(cm^{-1})
N-H symmetric stretching vibration	3749
Free OH stretch	3548
N-H symmetric stretching vibration	3417
Methyl C-H symmetric stretch; Methylene C-H asymmetric stretch	2954
CH2 bending	2877
N-H stretching	1828
Ester C =O stretch	1704
Methylene C-H symmetric bend, Methyl C-H symmetric bend	1458
CH3 wagging	1388
CH2 bending	1265
>CO-CH3 stretching vibration	1064

Inference:

The interpretation of IR spectra shown in Table no.14, 15 and 16. No interaction was observed between Simvastatin and Chitosan. They are compatible.

Formulation:

Simvastatin nanoparticles were prepared by Nanoprecipitation method using chitosan as a polymer with various ratios of drug and polymer. Formed Nanoparticles were separated by centrifugation.

Simvastatin loaded Chitosan nanoparticles

Fig.19: Simvastatin nanoparticles

CHARACTERISATION

Table.17

S.No	Formulation	Entrapment efficiency (%)	Percentage yield (%)	Loading efficiency	Drug content
1.	F1	89.15	25.16	16.3	32.68
2.	F2	95.56	44	14.8	44.40
3.	F3	97.43	52	17.07	68.32
4.	F4	93.85	32.9	6.02	30.12

ENTRAPMENT EFFICIENCY

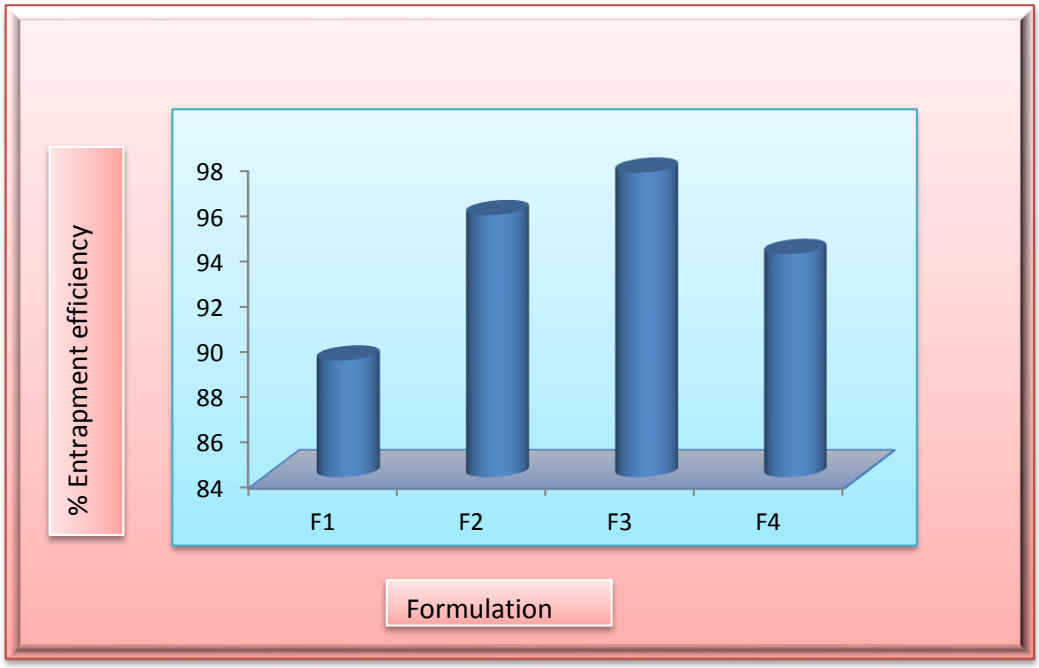


Fig.20: Entrapment efficiency

Inference

High chitosan concentration (4mg/ml) resulted in lesser encapsulation. Low chitosan concentration (1mg/ml) resulted in aggregates. The formation of nanoparticle was only possible within moderate concentration of chitosan. Chitosan solution within the concentration of (3mg/ml) produced opalescent nanoparticle suspension. Further increase in Chitosan concentration lead to decrease in encapsulation efficiency of Simvastatin nanoparticles. Chitosan concentration (such as 3mg/ml) promote the encapsulation of the drug.

PERCENTAGE YIELD

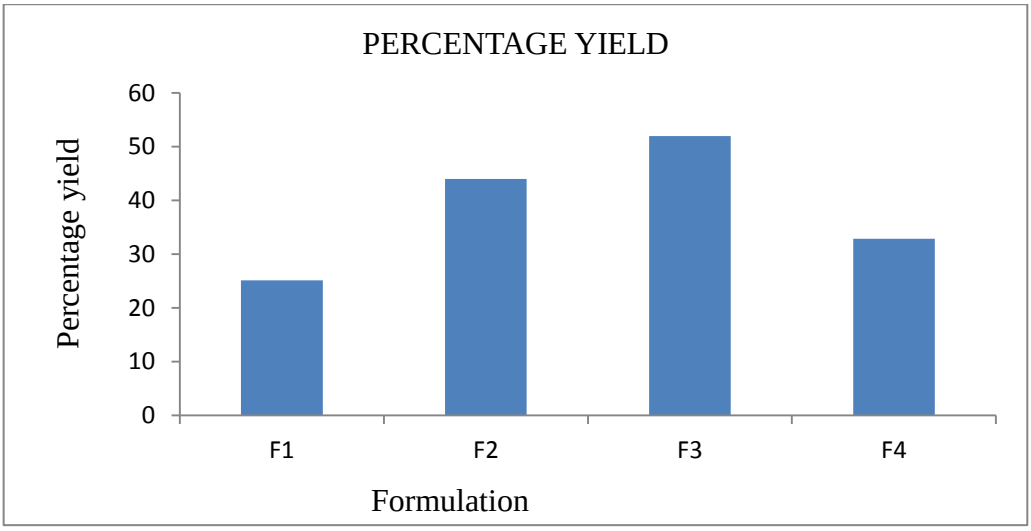


Fig.21: Percentage yield

Inference

1:3 ratio showed better yield shown in Fig.21 compared to other three ratios. Increased drug entrapment causes increased yield. It was assumed that the high entrapment of Simvastatin was due to its poor aqueous solubility, high binding of drug & polymer in organic phase and increased polymer ratio.

LOADING EFFICIENCY

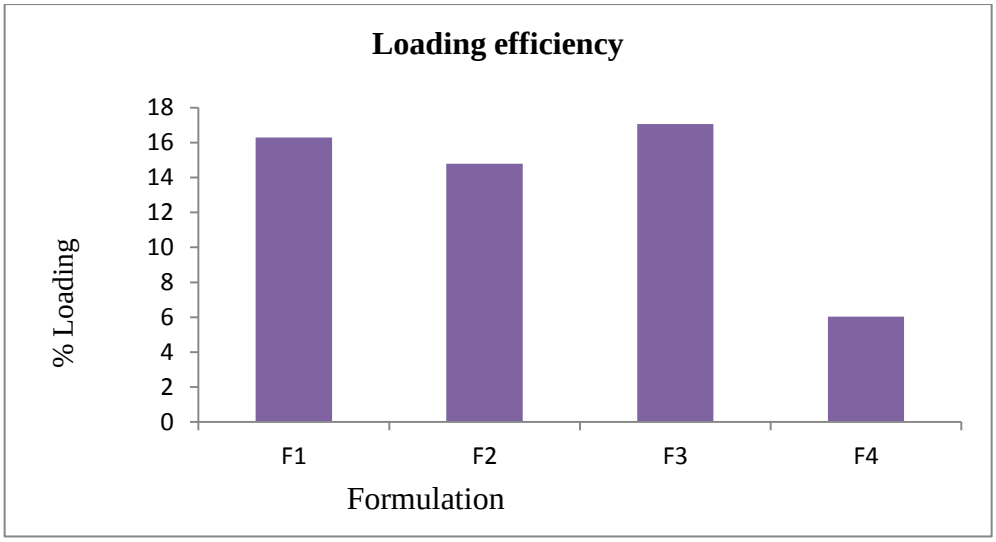


Fig.22: Loading efficiency

Inference

Formulation F3 showed highest dug loading capacity due to increasing polymer concentration.

DRUG CONTENT

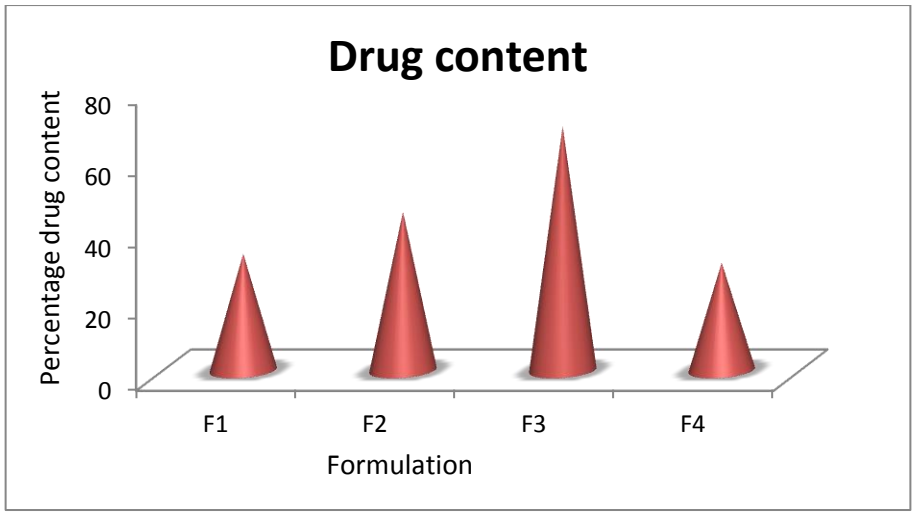


Fig.23: Drug content

Inference

The particle size of the nanoparticles was increased by increasing drug polymer ratio. Due to increased particle size, the drug content also increased from 32.68% to 68.32% as on increasing drug/polymer ratio from 1:1 to 1:3. There was no significant increase in drug content as polymer concentration was increased. This was probably caused by the increasing viscosity and hence poor dispersibility of Chitosan solution into the aqueous phase.

Particle size and Zeta potential of nanoparticles

Table.18: Particle size and Zeta potential

S.No	Batch.code	Polydispersity index	Average size (nm)	Zeta potential (mv)
1	F1	0.324±0.017	200±1.18	+16.3±1.6
2	F2	0.286±0.02	257±2.27	+17.6±1.8
3	F3	0.241±0.016	398±1.13	+19.8±2.2
4	F4	0.232±0.012	360±1.24	+20.5±1.6

Inference

Particle size is usually used to characterize the nanoparticles, because it facilitates understanding of dispersion and aggregation. Larger surface area and attractive forces between particles accompanies more aggregation of particles. The result showed that the average size of prepared nanoparticles varies from 200±1.18 to 398±1.13 shown in Table.18 with a polydispersity index in the range of 0.232+0.012 to 0.364+0.013. As the amount of polymer increased, size of the nanoparticles also increased. It was reported that polydispersity index more than 0.5 is indicative to aggregation of particles.

Zeta potential of prepared nanoparticles was found to range between +16.3±1.6 to +20.5±1.6mV. It was found that higher the zeta potential, less will be the particle aggregation, due to electric repulsion and hence more will be the stability of nanoparticles. It was observed that positive charge appear on nanoparticles surface which is attributed to the presence of the quaternary ammonium groups of chitosan.

SEM image of pure Simvastatin

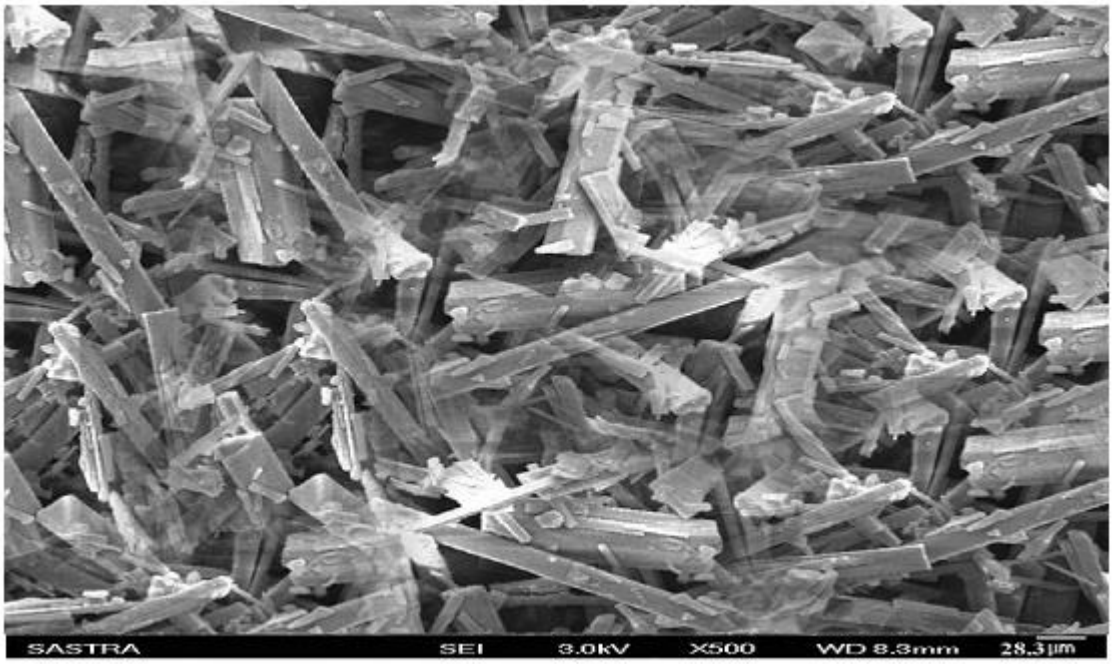
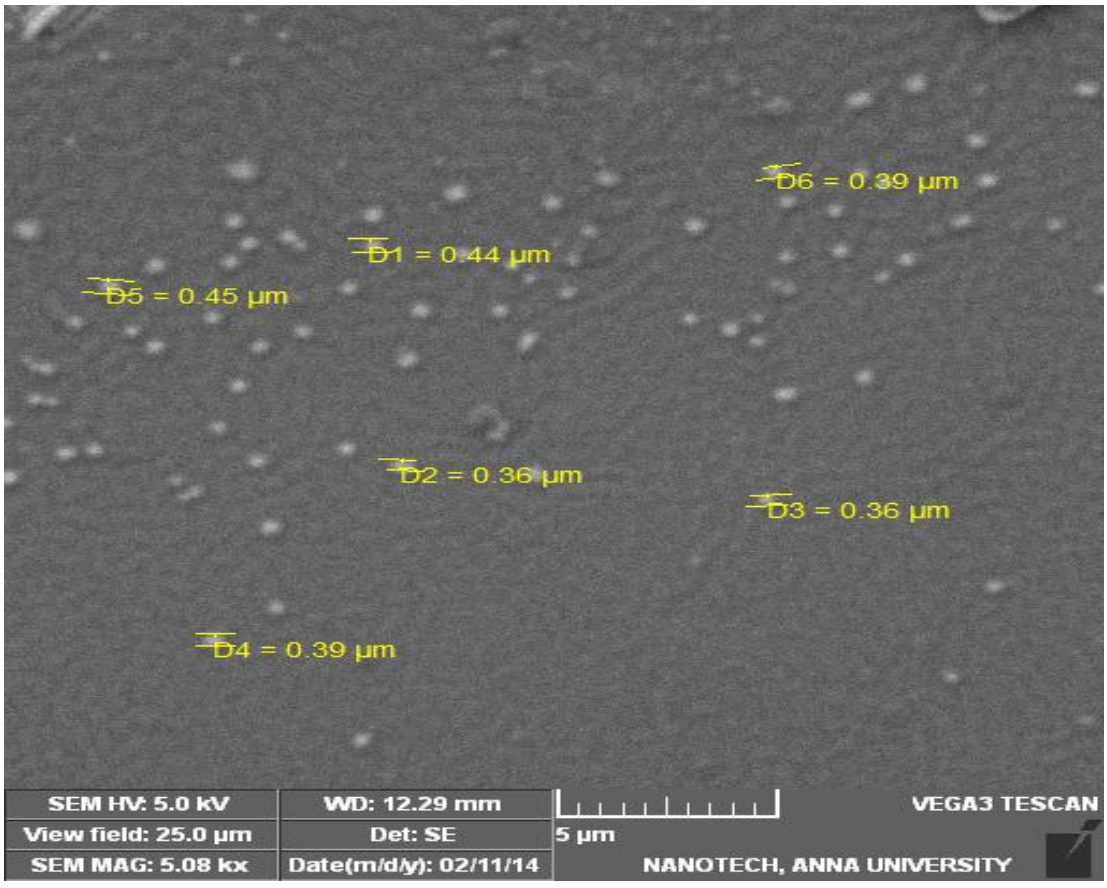


Fig.24: SEM image of Simvastatin

SEM image of Simvastatin nanoparticle- Fig.25: SEM image of Simvastatin Nano



Inference

The prepared nanoparticles were spherical in shape with a smooth surface. Particle size of prepared nanoparticles was found to be in the range of 360nm to 480nm shown in Fig.25. Pure simvastatin used for the study was characterized by relatively large particles with average value of about 28.3 µm as reported sown in Fig.24. The nanoparticles showed a drastic decrease in the particle size when compared to the pure drug particles. As per Noyes-Whitney equation, the decrease in the particle size will have a positive effect on the drug dissolution rate. Hence this decrease in the particle size achieved will have a significant effect in the drug solubility and dissolution characteristics.

Differential Scanning Colorimetry

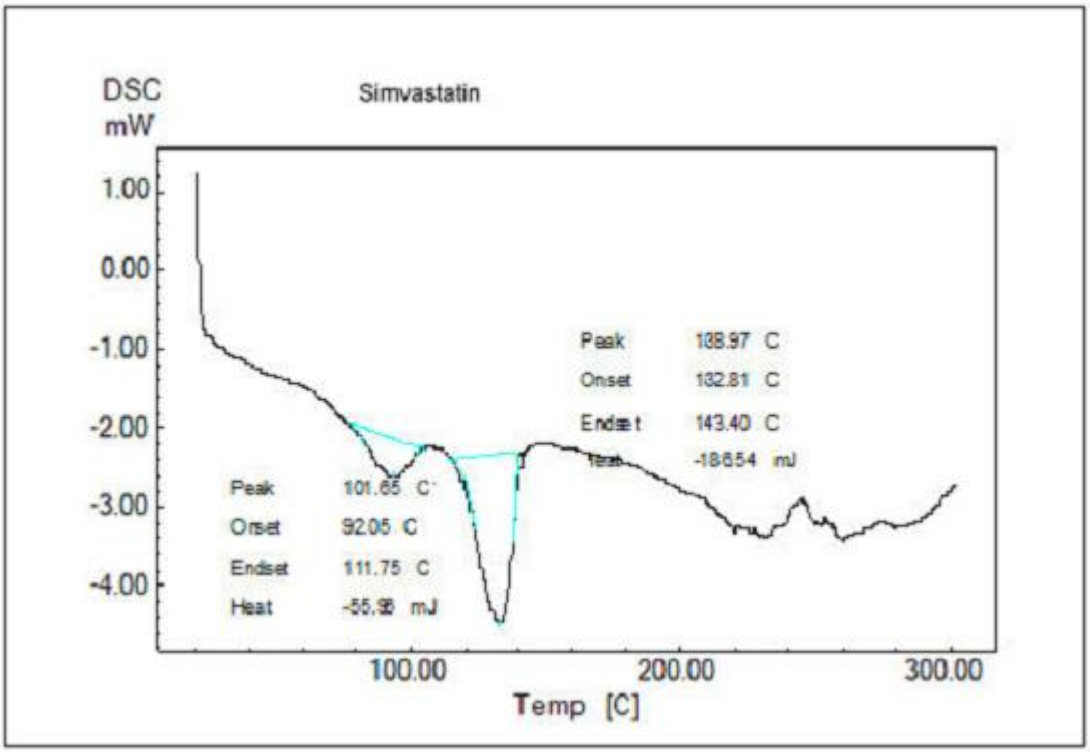


Fig.26: DSC Thermogram of pure drug (Simvastatin)

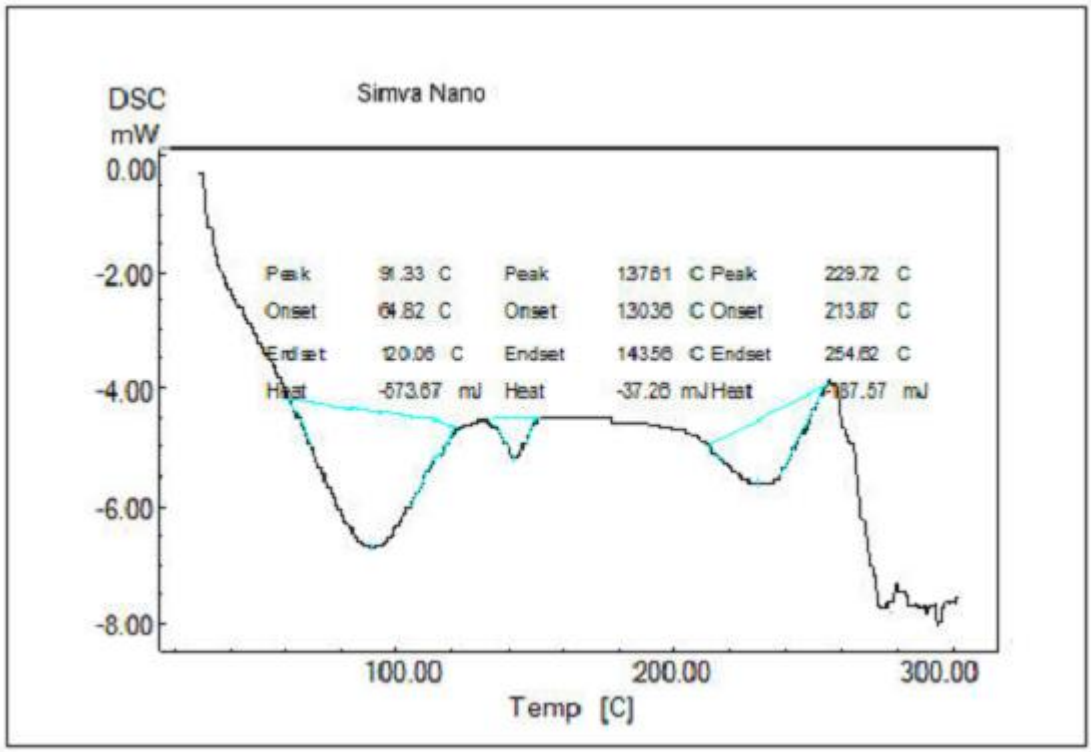


Fig.27: DSC Thermogram of Simvastatin Nanoparticles

Inference

The DSC curves of commercial simvastatin showed a broad endotherm ranging from 30 to 120°C indicating the loss of water and the sharp endotherm at 138.97°C might be due to the melting point of simvastatin shown in Fig.26, 27. There was no significant change in the melting points of the Formulation F3. Hence it is inferred that the drug and the polymer is compatible with each other.

FTIR of Simvastatin nanoparticle

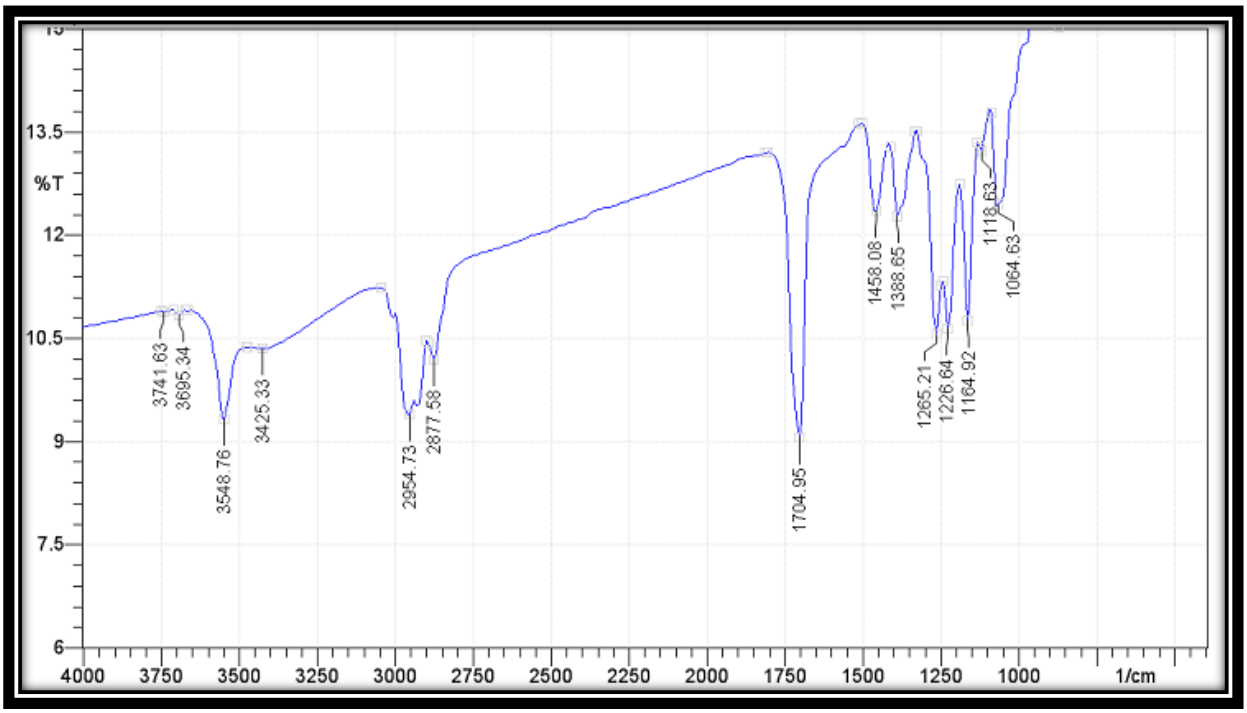


Fig.27: FTIR of Simvastatin Nanoparticle

FTIR data of Simvastatin nanoparticle- Table.19

Interpretation	Wave number(cm^{-1})
N-H symmetric stretching vibration	3741
N-H symmetric stretching vibration	3695
Free OH stretch	3548
N-H symmetric stretching vibration	3425
Methylene C-H asymmetric stretch	2954
CH2 bending	2877
Ester C =O stretch	1704
Methyl C-H symmetric bend	1458
CH3 wagging	1388
CH2 bending	1265
Lactone-C-O-C bend	1226
Confirmed a saccharide structure	1164
Secondary alcohol C-O stretch	1118
>CO-CH3 stretching vibration	1064

Inference

It was evident that all the characteristic peaks of Chitosan and Simvastatin were also in the formulation F3. Hence it was concluded that the drugs were compatible with the polymer, shown in Fig.27 and Table.19.

X-RAY DIFFRACTION STUDY

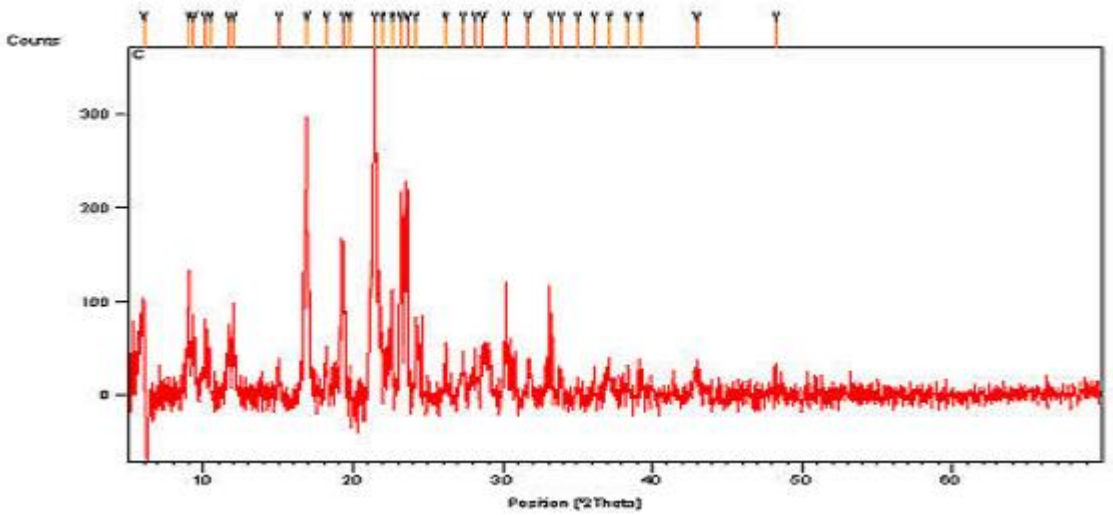


Fig.28: XRD Pattern of Pure Drug (Simvastatin)

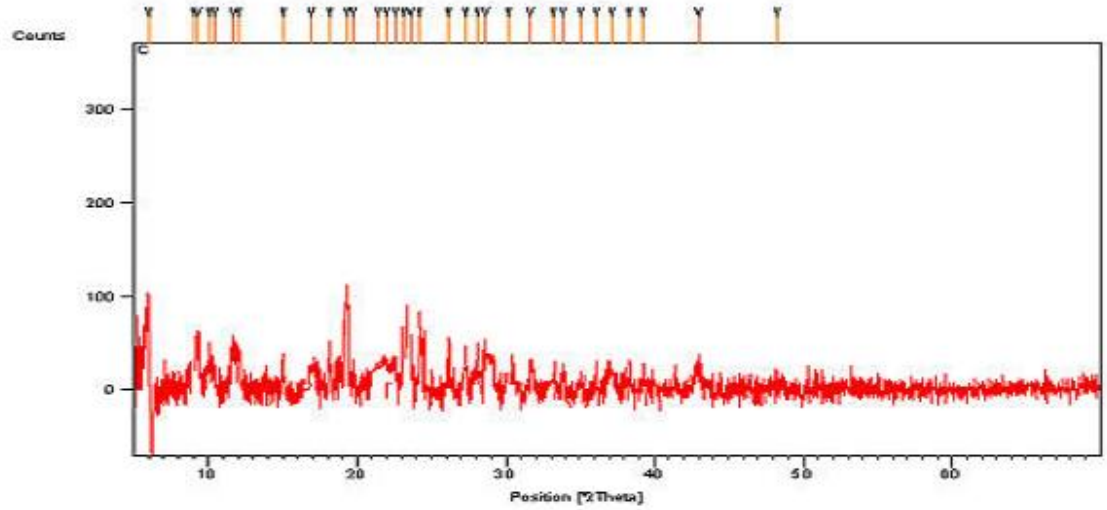


Fig.29: XRD Pattern of Simvastatin Nanoparticles

Inference

XRD pattern of the pure drug and selected formulation are showed Characteristic diffraction peaks were observed for commercial simvastatin. On the other hand, the nano formulations particles prepared with Chitosan was characterized by less intensity of the diffraction peak when compared to that of simvastatin. This clearly indicates the reduction in the crystallanity of the precipitated simvastatin nanoparticles shown in Fig. 28 and 29.

***In-vitro* drug release**

Table.20: *In-vitro* drug release*

Time(Hours)	Control	F1	F2	F3	F4
1	45.45±1.20	46.53±1.02	25.22±0.23	19.71±1.05	10.97±0.61
2	52.60±0.10	51.67±0.26	28.08±1.03	24.28±1.24	13±0.42
3	67.58±1.02	59.96±1.32	29.98±1.25	28.50±1.15	15.58±0.20
4	79.33±0.25	75.79±1.02	32.98±0.12	38.56±1.10	17.80±0.81
5	87.80±0.10	82.24±0.29	35.88±0.19	47.27±0.12	19.94±1.22
6	95.26±0.78	90.90±0.23	38.15±0.15	55.26±0.29	22.07±1.34
7		96.09±1.28	40.30±1.14	65.90±1.16	24.72±1.82
8			42.85±1.28	76.94±1.16	26.53±1.13
9			46.14±1.56	84.79±0.73	27.92±1.55
10			48.80±1.64	87.12±0.20	29.12±1.51
11			52.13±0.10	91.34±0.45	32.89±1.87
12			56.78±1.22	95.66±0.98	37.21±1.22
24			79.88±1.01		43.43±1.13

*Mean± S.D (n=3)

COMPARATIVE INVITRO DRUG RELEASE FOR ALL FORMULATION

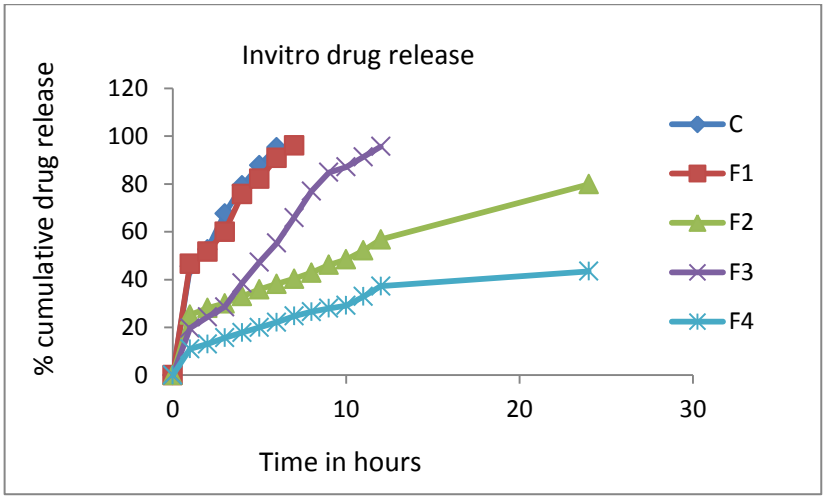


Fig.29: Comparative invitro drug release

Inference

The formulation F3 showed sustained release of drug shown in Table.20 and Fig.29.

In vitro drug release of the optimized formulation F3

Table.21: *In-vitro* drug release of optimised formulation*

S.No	Time(hours)	% cumulative drug release
1.	0	0
2.	1	19.71±0.61
3	2	24.28±0.42
4.	3	28.50±0.20
5.	4	38.56±0.81
6.	5	47.27±1.22
7.	6	55.26±1.34
8.	7	65.90±1.82
9.	8	76.94±1.13
10.	9	84.79±1.55
11.	10	87.12±1.51
12.	11	91.34±1.28
13.	12	95.66±0.73

***In-vitro* drug release of the optimised formulation**

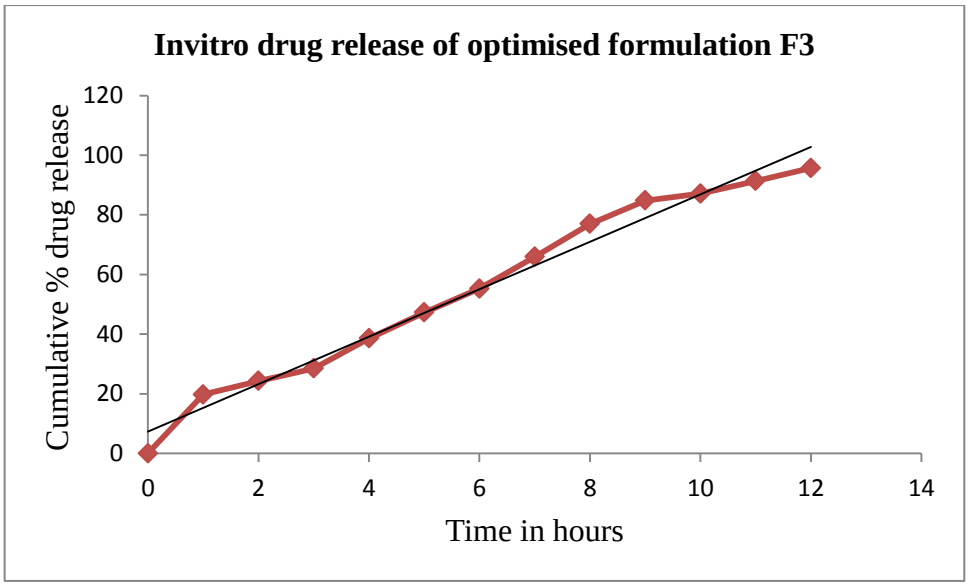


Fig.30: *In-vitro* drug release

Inference

The cumulative % drug release of the formulation F3 was found to be 95.66% at the end of 12 hours shown in Fig.30.

Data for release kinetics

Table.22: Data for release kinetics

Time(hours)	% cum drug release	%cum drug remaining	Log % cum drug remaining	Square root of time	Log time	Log % cum drug release	Cube root of % drug remaining
0	0	100	2	0	-∞	-∞	4.641
1	19.71	80.29	1.904	1.00	0	1.294	4.314
2	24.28	75.72	1.879	1.414	0.301	1.385	4.230
3	28.50	71.50	1.854	1.732	0.477	1.454	4.150
4	38.56	61.44	1.788	2.00	0.602	1.586	3.945
5	47.27	52.73	1.722	2.236	0.698	1.674	3.749
6	55.26	44.74	1.650	2.449	0.778	1.742	3.550
7	65.90	34.10	1.532	2.645	0.845	1.818	3.242
8	76.94	23.06	1.362	2.828	0.903	1.886	2.846
9	84.79	15.21	1.182	3	0.954	1.928	2.477
10	87.12	12.88	1.109	3.162	1	1.940	2.344
11	91.34	8.66	0.937	3.316	1.041	1.960	2.053
12	95.66	4.34	0.637	3.464	1.079	1.980	1.631

Determination of drug release mechanism of optimized formulation F3.

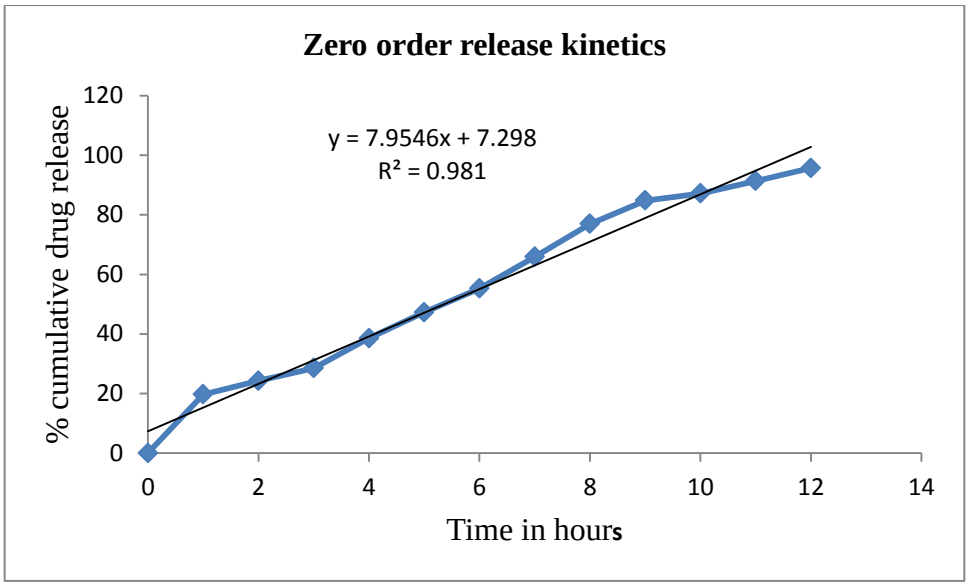


Fig.31: Zero order release kinetics

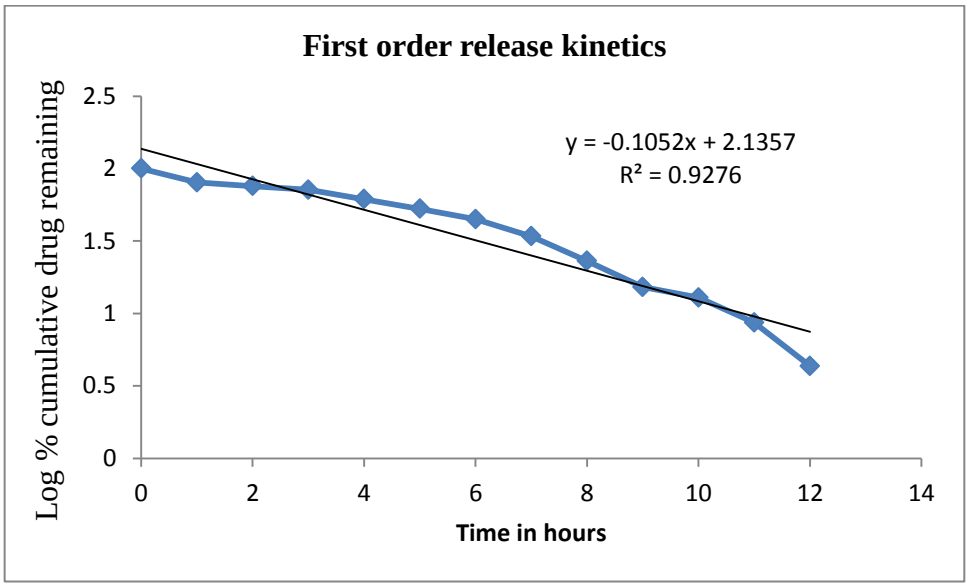


Fig.32: First order release kinetics

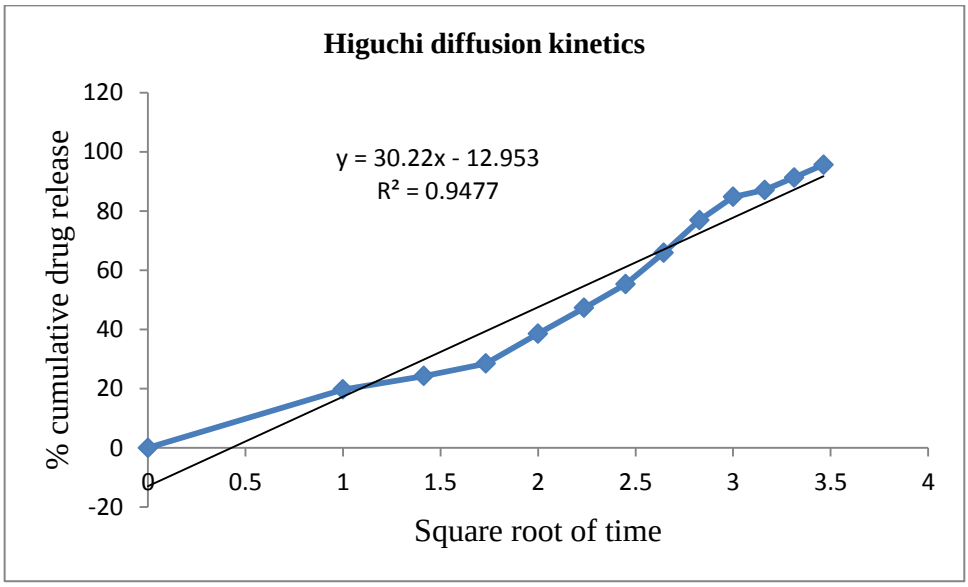


Fig.32: Higuchi diffusion kinetics

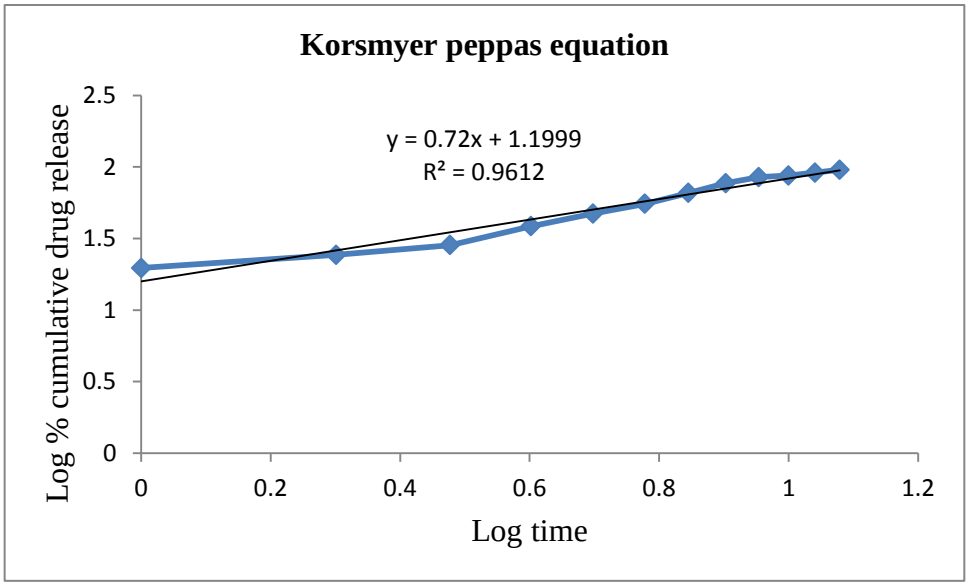


Fig.33: Korsmeyer Peppas equation

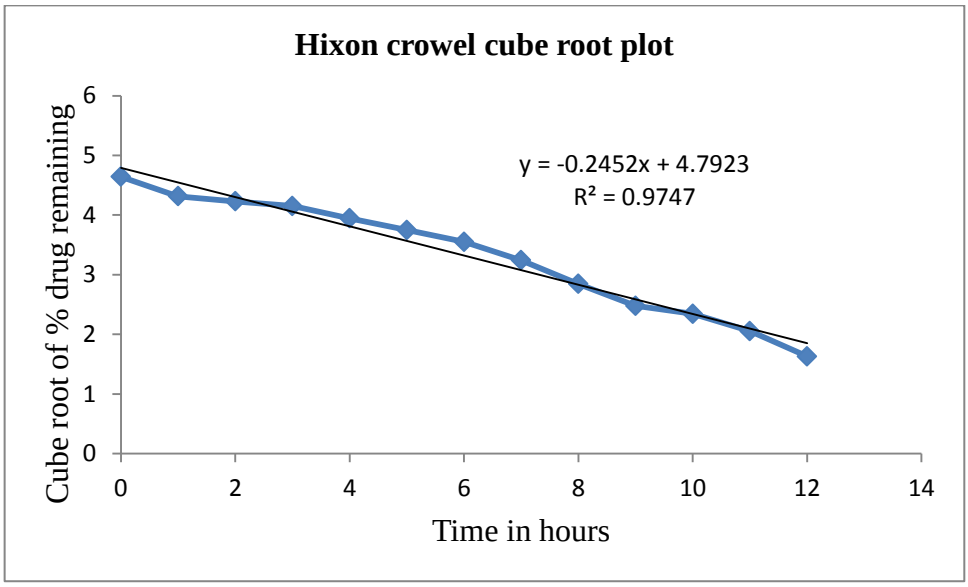


Fig.34: Hixon crowel cube root plot

***In-vitro* release kinetics**

Table.22: Release kinetics

S.No	Release kinetics	X-axis	Y-axis	Slope	Intercept	R^2	Linear equation
1.	Zero order equation	Time in hours	Cumulative % drug release	7.9546	7.298	0.981	y=7.9546x+ 7.298 R2=0.981
2.	First order equation	Time in hours	Log cum % drug remaining	-0.1052	2.1357	0.927 6	y = -0.1052x + 2.1357 R² = 0.9276
3.	Higuchi kinetics	Square root of time	Cum % drug release	30.22	0.7386	-12.95	y = 30.22x – 12.953 R² = 0.9477
4.	Korsmeyer Peppas equation	Log time	Log cum % drug release	0.72	1.199	0.961 2	y = 0.72x + 1.199 R² = 0.9612
5.	Hixon crowel cube root equation	Time in hours	Cube root % drug remaining	-0.2452	4.7923	0.974 7	y = -0.2452x + 4.7923 R² = 0.9747

Determination of drug release mechanism of optimized nanoparticle

- The order of release was found to be zero order, in which R^2 value was close to 1. So the formulation follows zero order kinetics. It also followed Higuchi release model
- The n value of Korsmeyer peppas equation was found to be greater than 0.5. It was concluded that the release follows a non-fickian diffusion.
- Good correlation coefficient was obtained for the kinetics parameters based on Higuchi's square root equation and it was found that release follows diffusion kinetics mechanism.

Inference

Drug may be released from nanoparticles by the mechanism of diffusion. It was observed that the mechanism governing release of Simvastatin from chitosan based nanoparticles is predominantly drug diffusion.

Stability studies of the optimized Formulation- F3

Table.23: Stability studies of optimised formulation- F3

Temperature	**Amount of drug retained (%) after months**			
	Initial	**I**	**II**	**III**
Refrigeration ($4^0 \pm 1^0$C)	82.99±1.10	80.78±0.20	77.90±0.95	75.46±1.23
Room Temperature	82.99±1.50	78.34±1.76	73.88±0.10	70.66±0.56
$40^0 \pm 2^0$C RH-70±5%	82.99±1.45	74.89±1.89	66.67±0.65	61.10±.90

***Mean± SD (n=3)**

Inference:

Stability studies for Simvastatin nanoparticles were carried out at various temperatures – refrigeration (4˚C), normal room temperature (25˚C) and stability chamber (40˚C/75% RH). Simvastatin Nanoparticles stored under refrigerated condition showed 75.46% after 3 months.

Simvastatin nanoparticles stored at room temperature the drug retained after 3 months was 70.66%. Simvastatin nanoparticles stored at $40^0 \pm 2^0$C/ 70±5% RH was 61.10% after 3 months shown in Table.23.

- The formulations were found most stable at refrigeration temperature
- Good stability was observed at Room Temperature
- Drug degraded at $40^0 \pm 2^0$C/70±5% RH.

***In-vivo* Study**

Hypercholesterolemia was induced in rats by feeding a high cholesterol diet. From days 0 to 21, four groups of albino rats were administered nanoparticle dispersion (equivalent to 10 mg/kg dose). The results were compared with the group of animals treated with reference treatment group as well as two control groups, one group serving as normal control, while the other group served as cholesterol control.

WEIGHT OF THE ANIMALS (g)

Table.24: Weight of the animals (g)

Groups	0 day	5th day	10th day	15th day	21st day
CTG	110	115	120	130	150
HTG	100	120	130	140	160
RTG	100	105	110	115	130
TTG	100	100	100	100	110

Inference

After 21 days of treatment with 2% coconut oil, 2% cholesterol and 1% sodium cholate per day, the body weight of rats in CTG, HTG, RTG and TTG groups was increased (26.66%, 37.50%, 23.07% and 9.09% respectively). The increase in body weight of rats in TTG is very marginal shown Table.24.

CHOLESTEROL LEVEL (mg/dl) **

Table.25: Cholesterol level

Groups	0 day	5th day	10th days	15th days	21st days
CTG	61.01±1.08	61.48±0.555	63.46±0.617	67.44±0.574	73.45±0.852
HTG	61.43±0.554	71.47±0.579	101.41±0.562	107.85±0.582	127.01±0.674
RTG	59.42±0.937	63.47±0.884	91.49±0.751	103.61±0.536	114.84±0.875
TTG	58.46±0.522	60.52±0.947	84.49±0.617	86.53±1.457	98.34±0.596

CTG- Control treatment groups, HTG- Hyperlipidemic control groups, TTG- Test treatment groups, RTG- Reference treatment groups, Significant differences p<0.01
****Mean ±SD (n=6)**

Inference

HTG, RTG showed a marked increase in total CH (127.01±0.674mg/dl, 114.84± 0.875 mg/dl respectively) .TTG showed an increase of 98.34 ± 0.596mg/dl ($p < 0.01$). Particularly, in the case of HTG a significant increase in CH ($p < 0.01$) shown in Table.25, from day zero to 21 of the treatment, indicated the inducement of hypercholesterolemia due to administration of (2% coconut oil, 2% cholesterol and 1% sodium cholate). There was a significant difference in the tested parameters for CTG and HTG after the 21 day treatment on the lipid profiles of experimental animals.

TRIGLYCERIDES LEVEL (mg/dl) **

Table.26: Triglycerides level

Groups	0day	5th day	10th day	15th day	21st day
CTG	23.53±0.595	25.60±0.618	49.44±0.579	53.31±1.058	56.92±0.630
HTG	24.27±0.542	36.54±0.525	65.21±0.532	99.44±0.807	185.84±1.070
RTG	22.51±0.957	29.65±0.673	55.38±0.578	81.59±0.571	101.41±1.219
TTG	22.47±0.899	24.50±0.619	44.39±0.626	62.53±0.817	84.56±0.550

****Mean ±SD (n=6)**

Inference

HTG, RTG showed a marked increase in total TG (185.84±1.070mg/dl, 101.41± 1.219mg/dl respectively). TTG showed an increase of 84.56±0.550mg/dl ($p < 0.01$). Particularly, in the case of HTG a significant increase in TG ($p < 0.01$) shown in Table.26 from day zero to 21 of the treatment was observed.

HIGH DENSITY LIPOPROTEINS (mg/dl) **

Table.27: HDL level

Groups	0day	5th day	10th day	15th day	21st day
CTG	24.26±0.602	24.49±0.520	25.53±0.526	25.57±0.629	31.37±0.608
HTG	24.49±0.585	23.56±0.650	21.44±0.691	20.49±0.597	19.41±0.606
RTG	23.52±0.621	24.39±0.591	25.32±0.754	26.49±0.570	28.80±0.791
TTG	22.50±0.795	24.31±0.582	26.62±0.598	29.19±0.530	36.29±0.602

****Mean ±SD (n=6)**

Inference

HTG showed a marked decrease in total HDL-CH (19.41±0.606mg/dl) contrast to increasing HDL-CH for RTG and TTG (28.80±0.791mg/dl, 36.29±0.602mg/dl respectively) ($p < 0.01$) shown in Table.27. Particularly, in the case of HTG a significant decrease in TG ($p < 0.01$), from day zero to 21 of the treatment was observed.

LOW DENSITY LIPOPROTEINS (mg/dl) **

Table.28: LDL level

GROUPS	0day	5th day	10th day	15th day	21st day
CTG	32.04±1.381	31.87±0.937	28.10±0.954	31.87±1.245	30.69±0.563
HTG	32.09±0.966	40.60±0.753	66.97±1.137	65.09±2.200	70.49±0.024
RTG	31.39±1.485	33.12±1.380	55.08±0.737	60.80±1.135	65.76±0.779
TTG	31.47±0.454	31.25±0.828	48.99±1.292	45.83±1.065	45.13±0.297

****Mean ±SD (n=6)**

VERY LOW DENSITY LIPOPROTEINS (mg/dl) **

Table.29: VLDL level

GROUPS	0 day	5th day	10th day	15th day	21st day
CTG	4.70±0.119	5.11±0.124	9.86±0.11	9.99±0.729	11.38±0.126
HTG	4.85±0.108	7.305±0.104	12.99±0.112	19.78±0.206	36.99±0.343
RTG	4.50±0.192	5.926±0.134	11.07±0.115	16.31±0.11	20.27±0.243
TTG	4.49±0.178	4.89±0.123	8.87±0.125	12.50±0.164	16.90±0.111

****Mean ±SD (n=6)**

Inference

In TTG, the increase in LDL-CH and VLDL were (45.13±0.297mg/dl, 16.90±0.111, respectively). The increase in LDL-CH and VLDL for CTG (30.69±0.563mg/dl, 11.38±0.126 respectively) $p<0.01$, shown in Table 28 and 29.

ATHEROGENIC INDEX **

Table.30: Atherogenic Index

GROUPS	0day	5th day	10th day	15th day	21st day
CTG	-0.01±0.021	0.01±0.016	0.28±0.010	0.31±0.017	0.25±0.011
HTG	-0.003±0.019	0.190±0.014	0.48±0.015	0.691±0.009	0.98±0.015
RTG	-0.019±0.014	0.084±0.013	0.34±0.010	0.488±0.007	0.54±0.009
TTG	-0.001±0.005	-0.001±0.012	0.22±0.007	0.357±0.023	0.36±0.009

****Mean ±SD (n=6)**

Inference

The AI increased in TTG (0.36±0.009mg/dl), When compared with CTG (0.25±0.011mg/dl) $p<0.01$. The AI decreased in TTG (0.36±0.009mg/dl) when compared with HTG and RTG (0.98±0.015mg/dl, 0.54±0.009mg/dl respectively) $p<0.01$ shown in Table.30.

Thus, Simvastatin-loaded chitosan nanoparticles showed a significantly better *in- vivo* performance than Simvastatin in reducing total CH and TG levels which is primarily attributed to the improved solubility and dissolution of nanoparticles. The present results suggest that HMG-CoA reductase inhibitors prevent the progression of hypercholesterolemia during treatment, though the plasma lipid levels remain much higher than in normal lipidemic rats. This may be due to the decreased HMG-CoA reductase activity and LDL receptor function in chronically fed cholesterol rats. The decrease in plasma lipid levels was more in TTG than in RTG. This may be due to the ability of Simvastatin to increase lipoprotein lipase activity in animals.

SUMMARY AND CONCLUSION

11. SUMMARY AND CONCLUSION

The present work involves the formulation development, optimization, *in-vitro* and *in-vivo* evaluation of Simvastatin loaded Chitosan nanoparticles for sustained release.

- The Simvastatin, Chitosan were found to be compatible in FTIR study and DSC studies.

- The Simvastatin Nanoparticles were prepared by Nanoprecipitation method.

- Various concentration of chitosan were used to prepare Simvastatin nanoparticles and the maximum drug loading was found in F3.

- SEM study showed that prepared nanoparticles were spherical in shape with a smooth surface. Particle size of prepared nanoparticles was found to be in the range between 360nm and 480nm.

- *In-vitro* drug release of the optimized formulation shows 95.66% release at the end of 12 hours.

- The release kinetics of the optimized Nanoparticles showed that it follows zero order release kinetics. The release of the drug from Nanoparticles was found to be by diffusion and Non-fickian release.

- Stability studies of optimized nanoparticles were carried out according to ICH guidelines. It indicated that the Nanoparticles are stable and does not show any significant changes physical characteristics, drug content and dissolution.

- The comparative *in vivo* hypolipidemic activity performed on albino rats, after 21days of treatment, showed that plasma CH and TG levels were significantly lower (98.34±0.596 mg/dl, 84.56±0.55 mg/dl respectively) ($p < 0.01$).

- HDL-CH levels were significantly higher (36.29±0.602 mg/dl, $p < 0.01$) in TTG compared to RTG.

- ❖ TTG showed a significantly better *in vivo* performance than RTG in terms of plasma lipid profile. The maximum percentage reduction of lipid levels were observed with Simvastatin nanoparticles.

From the overall results, it is clear that the formulation F3 containing 1:3 drug: polymer ratio is the optimal formulation, as it produces sustained drug release.

FUTURE SCOPE

- Scale up studies of the optimized formulation.
- *In vivo – In vitro* correlation studies.
- Pharmacokinetic and toxicity study.

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