FORMULATION AND CHARACTERIZATION OF GELATIN LOADED ROSUVASTATIN NANOPARTICLES BY TWO STEP DESOLVATION METHOD

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In partial fulfillment for the award of Degree of
MASTER OF PHARMACY
(Pharmaceutics)

Submitted by
M.UDHAYA KUMAR
Register No .26106010

Under the Guidance of
Prof. K. SUNDARAMOORTHY, B.Sc., M. Pharm.,
Department of Pharmaceutics

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(Accredited by "NAAC" with a CGPA of 2.74 on a four point scale at "B"-Grade)
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CERTIFICATE

This is to certify that the research work entitled**"FORMULATION AND CHARACTERIZATION OF GELATIN LOADED ROSUVASTATIN NANOPARTICLES BY TWO STEP DESOLVATION METHOD"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the award of the Degree of the Master of Pharmacy (Pharmaceutics) was carried out by **M.UDHAYAKUMAR (Register No.26106010)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2011-2012.

Place: Melmaruvathur

Date:

Prof. K. SUNDARAMOORTHY, B.Sc., M. Pharm.,
Department of Pharmaceutics,
Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603 319,
Tamilnadu.

CERTIFICATE

This is to certify that the dissertation entitle **"FORMULATION AND CHARACTERIZATION OF GELATIN LOADED ROSUVASTATIN NANOPARTICLES BY TWO STEP DESOLVATION METHOD".**The bonafide research work carried out by **M.UDHAYA KUMAR (Register No.26106010)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai under theguidance of **Prof. K. SUNDARAMOORTHY, B. Sc., M. Pharm.,** Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year

2011-2012.

Place: Melmaruvathur

Date:

Prof. Dr. T. VETRICHELVAN, M. Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603 319,
Tamilnadu.

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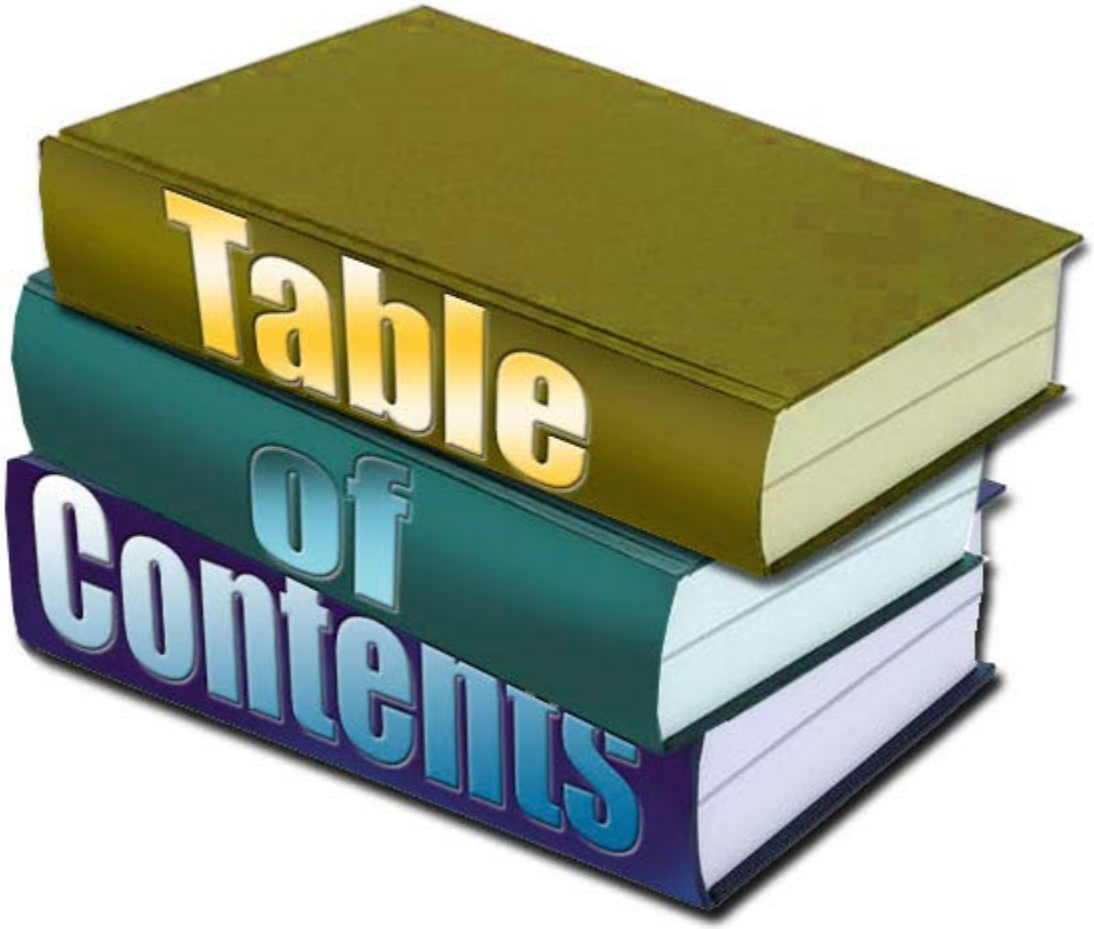
UDHAYA KUMAR.M

DEDICATED TO

MY BELOVED FAMILY

&

ALL MY FRIENDS...✍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LIST OF ABBREVIATIONS

%	-	Percentage
µg	-	Microgram
BBB	-	Blood Brain Barrier
BP	-	British Pharmacopoeia
C	-	Slope
Cps	-	Centi poises
CR	-	Controlled Release
CNT	-	Carban nanotubes
DSC	-	Differential Scanning Calorimetry
DNA	-	Deoxy nuclic acid
DTA	-	Differential Thermal Analysis
ENV	-	Envelope
EM		Eletromegnetic waves
F	-	Formulation
FTIR	-	Fourier Transform Infra-Red spectrum
FDA	-	Food Drug Administration
Gm	-	Gram
HMG-CO	-	Hydroxy methyl glutaryl coenzyme A
HCl	-	Hydro Chloric acid
nm	-	nanometer
ICH	-	International Conference on Harmonization

i.e	-	That is
IP	-	Indian Pharmacopoeia
IV	-	Intravenous
KBr	-	Potassium Bromide
LE	-	Loading Efficiency
LDL	-	Low dencity lipoprotein
LC	-	Loading Capacity
LOD	-	Loss On Drying
m	-	Slope, Units of response
MAC	-	Macro phase
ml	-	Milli litre
mg	-	Milligram
Mp	-	Melting point
MVB	-	Multivesscular bodies
N	-	Normality
nm	-	nanometer
NP	-	Nanoparticles
NDDS	-	Novel Drug Delivery System
pH	-	Negative Logarithm of hydrogen ion Concentration
Ppm	-	parts per million
PBS	-	Phosphate buffer saline
PCS	-	Photon correlation spectroscopy

PACA	-	Poly Alkyl-Cyanoacrylate
PLA	-	Poly Lactide
PLGA	-	Poly Lactide Co Glycolide
PMA	-	Polymethacrylate
PMMA	-	Polymethyl methacrylate
PVA	-	Polyvinyl alcohol
PVD	-	Physical Vapour Deposition
QIA	-	Quality improvement activity
RH	-	Relative Humidity
rpm	-	revolutions per minute
RES	-	Reticulo Endothelial System
Sol	-	Solution
S.D.	-	Standard Deviation
SEM	-	Scanning Electron Microscopy
SLN	-	Solid Lipid Nanoparticles
TEM	-	Transmission electron Microscopy
t	-	Time
$t_{1/2}$	-	Biological half-life
USP	-	United State Pharmacopoeia
UV-VIS	-	Ultraviolet-Visible Spectroscopy
VLDL	-	Very low dencity lipoprotein
λ max	-	Absorption maximum

INTRODUCTION

1.INTRODUCTION

1.1. Novel drug delivery system *(Shoba Rani., 2008)*

Recently, several technical advancements have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and or targeting the delivery of drug to a tissue. These are referred to as novel drug delivery systems. And they have revolutionized the method of medication, provides a number of therapeutic benefits.

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. 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.

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

- Desorption of surface-bound /adsorbed drugs;
- Diffusion through the carrier matrix;
- Diffusion (in the case of nanocapsules) through the carrier wall;
- Carrier matrix erosion.
- A combined erosion /diffusion process.

Advantages of novel drug delivery systems.(*Illinois., 2004)*

- Improve therapy by increasing the duration of action and reducing side effects.
- Increase patient compliance through decreased dosing frequency and convenient routes of administration
- Achieved targeting of to a specific site to reduce unwanted side effects and obtain maximum efficacy.
- Lead to reduction in dose and thus reduction side effects of drugs.
- Decreased toxicity/side effects and increased convenience.
- Shorter hospitalization and better patient compliance.
- Viable treatments for previously in arable disease.
- Potential prophylactic application

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. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature).

Novel drug delivery system is a system that offers multiple drug delivery solutions such as.

- ❖ Oral Drug Delivery Systems and Materials
- ❖ Parenteral and Implant Drug Delivery Systems
- ❖ Pulmonary and Nasal Drug Delivery
- ❖ Transmucosal Drug Delivery
- ❖ Transdermal and Topical Drug Delivery
- ❖ Delivery of Proteins and Peptides
- ❖ Drug Delivery Pipelines

Nanoparticles for drug delivery.

- ❖ Metal-based nanoparticles
- ❖ Lipid-based nanoparticles
- ❖ Polymer-based nanoparticles
- ❖ Biological nanoparticles

1.1.3. Methods of drug delivery.*(ChayaVenkat., et al., 2003)*

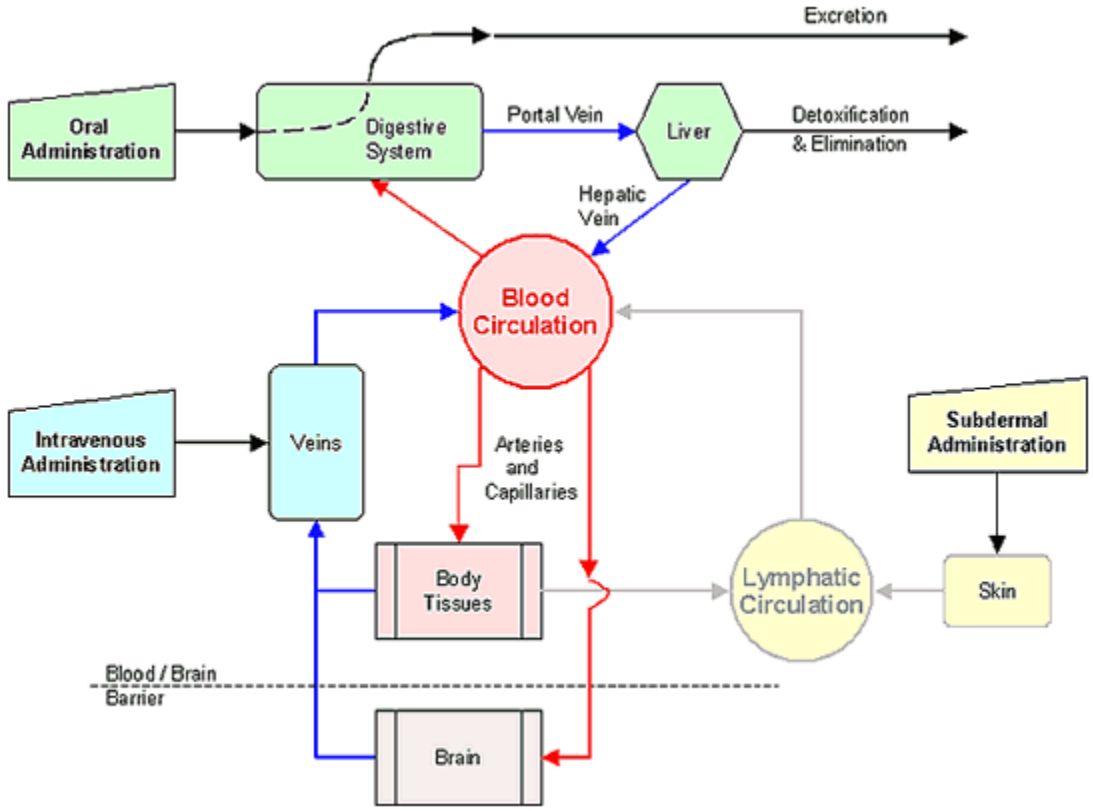


Figure 1.1: Methods of drug delivery

Types of novel drug delivery systems.*(* Costas Kaparissides*., et al., 2005)*

- Hydrogels
- Colloids
- Microspores
- Liposomes
- Nanoparticles
- Mucoadhesives
- Transdermal

Hydrogels.

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical crosslinks (tie-points, junctions), or physical crosslinks, such as entanglements or crystallites. They are used to regulate drug release in reservoir-based, controlled release systems or as carriers in swellable and swelling-controlled release devices.

Liposomes.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids.

Nanoparticles.

Nanoparticles(including nanospores and nanocapsules of size 10-200 nm) can adsorb or encapsulated a drug,thus protecting it from chemical & enzymatic degradation. Nanoparticles as drug carriers can be formed from both biodegradable and non-biodegradable polymer.

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.

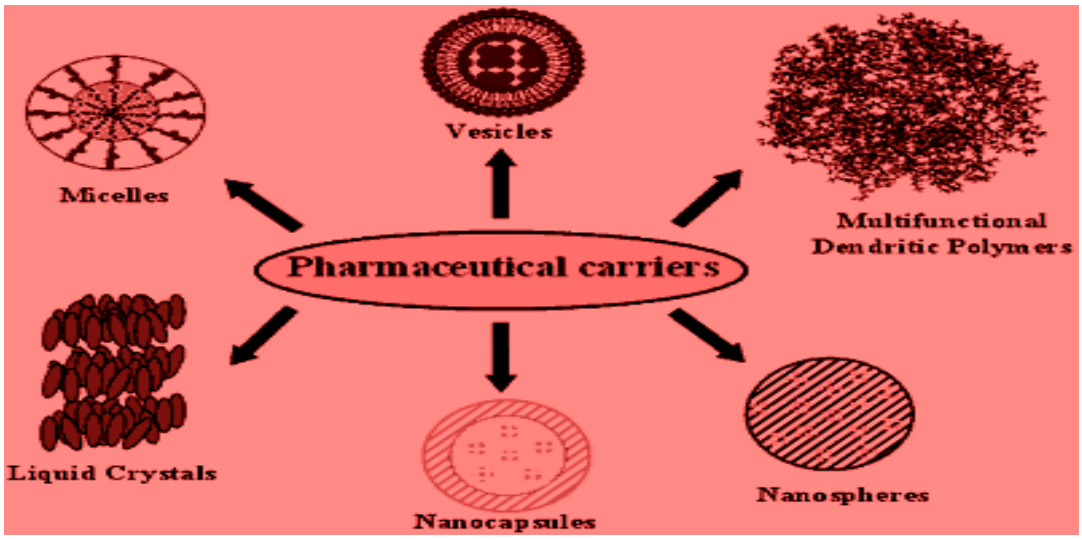


Figure 1.2: Pharmaceutical carriers

1.2. Nanotechnology. *(Shoba Rani., 2008)*

Definition of Nanoparticles.

Nanoparticles are solid, colloidal particles ranging 10-1000nm (1µm) in size. They consist of macromolecular materials in which the active ingredient (drug or biologically active material) is dissolved, entrapped or encapsulated, and or absorbed or attached.

Nanoparticles are often defined as particles of less than 100nm in diameter. Nanoparticles can be also defined as particles less than 100nm in diameter that exhibit new or enhanced size-dependent properties compared with larger particles of the same material.

Images of Nanoparticle

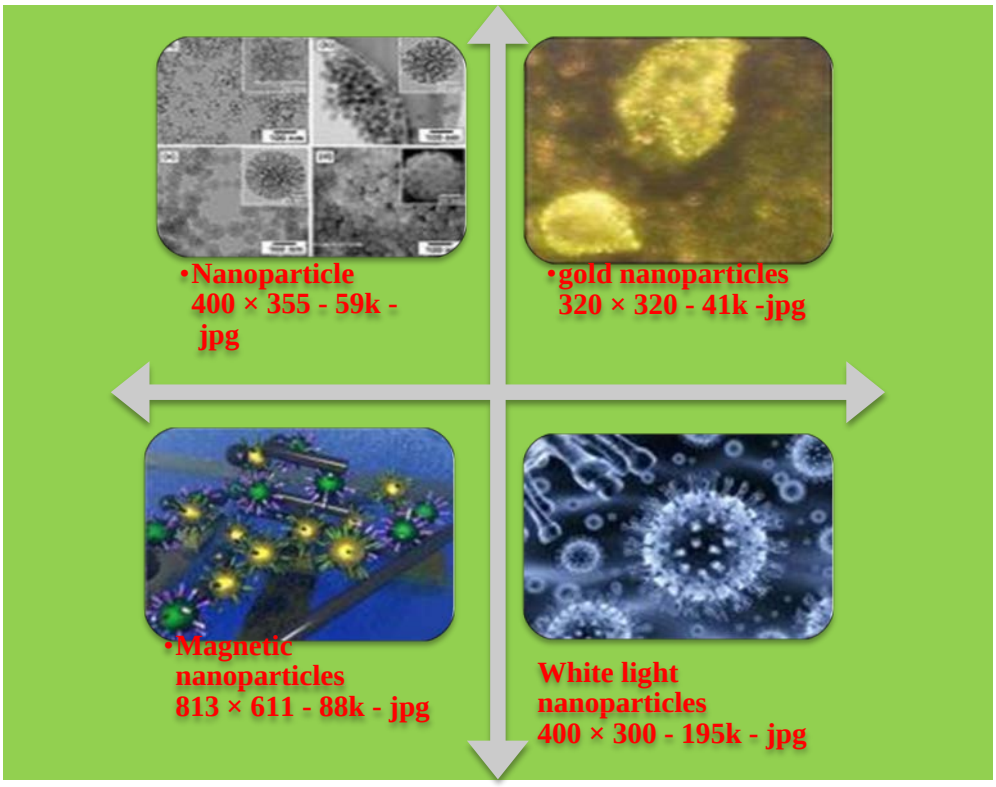


Figure 1.3: Images of Nanoparticle

How Nanoparticles are transported into cells (Krishnadasan., et al.,2004)

Nanoparticles, proteins and other macromolecules are taken up in the body's cells via various mechanisms. The nanoparticles are first encapsulated in fatty bubbles, called vesicles, which form on the cell surface.

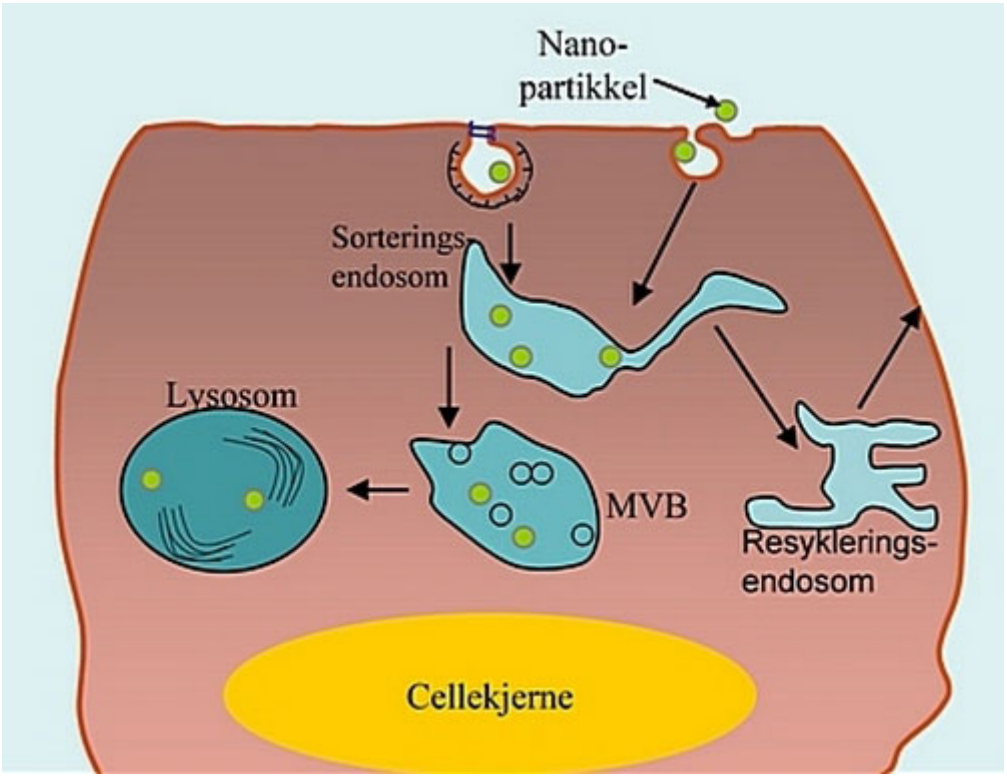


Figure 1.4: Mechanism

These vesicles merge with larger vesicles known as sorting endosomes, which can then mature into multivesicular bodies (MVBs). These MVBs, in turn, merge with lysosomes, where proteins and other macromolecules are broken down by proteases and other enzymes. Nanoparticles can be transported out of the cell via either sorting endosomes or recycling endosomes.

Milestones of Nanoparticles.*(Steffen Petersen., 2008)*

30BC-640AD - The Lycurgus Cup (an apparently accidental early use of nanotech) contains gold and silver nanoparticles which cause it to change color from green to red when illuminated from the outside or inside, respectively.

1450-1600 - Renaissance Artisans (Deruta Ceramicists) used copper and silver nanoparticles between 5 and 100 nm to produce metallic and iridescent glazes on ceramics.

1827 - Photography depends upon light sensitive nanoparticles to form photographic images.

1856 - Researcher, Michael Faraday discovers/prepares the first metallic colloids. His gold colloids (fine particles suspended in solution) had unique optical/electronic properties.

1959 - Richard Feynman delivers famous speech, ″There's Plenty of Room at the Bottom″

1960 - William McLellan constructs the first 250 microgram, 2000 rpm motor out of 13 individual parts, as large as the period at the end of this sentence.

1974 - Professor Norio Taniguchi becomes the first to formulate and use the term Nano-technology.

1985 - Fullerenes (commonly called buckyballs) are discovered by researchers at Rice University.

1986 - Gerd Binnig, Christopher Gerber and Calvin F. Quate invent the Atomic Force Microscope

1986 - Eric Drexler, an American Engineer and the founder of Foresight Nanotech Institute, writes Engines of Creation, introducing nanotechnology to the world.

1989 - The first commercially available Atomic Force Microscope is introduced.

1991 - Carbon nanotubes (CNT) are discovered by SumioIijima. Nanotubes are essentially rolled sheets of graphene which can be single or multi-walled. Nanotubes have potential applications in electronics, composite materials, the space elevator, and drug delivery.

1994 - US science advisor, Dr. Jack Gibbons talks at the White House about nanotechnology and calls for increased .

1997 - Zyvex, the first company to research nanotechnology, is founded.

1999 - Consumer nanotech-based products start appearing on the global marketplace.

2003 - Congress enacts the 21st Century Nanotechnology Research and Development Act.

2009 - The outlines a new research and regulatory strategy to better control the production and use of engineered nanomaterials.

2010 - The House of Lords Science and Technology Committee, United Kingdom, warns its country's food industry against hiding its use of nanotechnology.

Ideal properties of polymeric based Nanoparticles*(Deepak Thassu.,et al., 2007)*

- Natural or synthetic polymer
- Inexpensive
- Nontoxic
- Biodegradable
- Nonthrombogenic
- Nonimmunogenic

Nanoparticle recovery & drug incorporation efficiency

(*Leroueli Le Verger M., et al., 1998)*

$$\text{Nanoparticles recovery \%} = \frac{\text{Concentration of drug in Nanoparticles}}{\text{Concentration of Nanoparticles recovered}} \text{X100}$$

Drug incorporation efficiency has been expressed both as drug content (%w/w), also referred to as drug loading, & drug entrapment (%) represented by the following equation.

$$\text{Drug incorporation efficiency in Nanoparticles} = \frac{\text{Amount of drug entrapped in Nanoparticles}}{\text{Total amount of drug added}}$$

1.2.1. Characterization of Nanoparticles. *(Rakesh P. Patel.,et al., 2008)*

Table 1.1: Characterization of Nanoparticles

Parameters	Characterization methods
Particle size and size distribution	photon correlation spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM), Mercury porositometry, Laser defractrometry
Charge determination	Laser droplet anemometry, Zeta potentiometer
Surface hydrophobicity	Water contact angle measurements, rose bangle (dye) binding, hydrophobic interaction chromatography, X-ray photoelectron spectroscopy
Chemical analysis of surface	Static secondary ion mass spectrometry, sorptometer
Carrier drug interaction	Differential scanning calorimetry
Nanoparticle dispersion stability	Critical flocculation temperature(CFT)
Release profile	In-vitro release characteristic under physiologic & sink condition
Drug stability	Bioassay of drug extracted from nanoparticle, chemical analysis.

1.2.2. Classification of Nanoparticles.*(Rakesh P. Patel., et al., 2008)*

1**. In one dimensions** (Thin surface coatings)

One-dimensional systems, such as thin films or manufactured surfaces.

***2*. In Two dimensions.**

a) Carbon Nanotubes

Carbon nanotubes are a new form of carbon molecule. Wound in a hexagonal network of carbon atoms, these hollow cylinders can have diameters as small as 0.7 nm and reach several millimeters in length. Each end can be opened or closed by a fullerene half-molecule. These nanotubes can have a single layer (like a straw) or several layers (like a poster rolled in a tube) of coaxial cylinders of increasing diameters in a common axis.

3. In three dimensions.

a) Fullerenes (Carbon 60)

Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms displaying unique physical properties. They can be subjected to extreme pressures and regain their original shape when the pressure is released.

b) Dendrimers

Dendrimers represent a new class of controlled-structure polymers with nanometric dimensions. They are considered to be basic elements for large-scale synthesis of organic and inorganic nanostructures with dimensions of 1 to 100 nm, displaying unique properties. Compatible with organic structures such as DNA, they can also be fabricated to interact with metallic nanocrystals and nanotubes or to possess an encapsulation capacity

c) Quantum dots

It represents a special form of spherical nanocrystals from 1 to 10 nm in diameter. They have been developed in the form of semiconductors, insulators, metals, magnetic materials or metallic oxides.

Types of Nanoparticles

- Quantum Dots
- Nanocrystalline silicon
- Photonic
- Liposome
- Gliadin Nanoparticles
- Polymeric Nanoparticles
- Solid Lipid Quantum Nanoparticles
- Others-Gold.Carbon, Silver.etc.

1. Quantum dots

A quantum dot is a semiconductor nanostructure that confines the motion of conduction band electrons, valence band holes, or excitons (pairs of conduction band electrons and valence band holes)in all three spatial directions. A quantum dothas a discrete quantized energy spectrum. A quantum dot4 contains a small integer number of the order of 1-100) of conduction band electrons, valence band holes, or excitons

2. Nanocrystalline silicon.

Nanocrystalline silicon is anallotropic form of silicon – is similar to amorphous silicon is sometimes also known as microcrystalline silicon. One of the most important advantages of

Nanocrystalline silicon, it has increasdstabilithovara one of the reasons being because of its lower hydrogen concentration.

3.Photonic crystals.

Photonic crystals are periodic dielectric or metallo-dielectric (nano) structures that are designed to affect the propagation of electromagnetic waves (EM) in the same way as the periodic in a semiconductor crystal affects the electron motion by defining allowed and forbidden electronic energy bands. Photonic crystals are the attractive optical materials for controlling and manipulating the flow of light. They are of great interest for both fundamental & applied research, & are expected to find commercial applications soon.

4.Liposomes.

A Liposome is a spherical vesicle with a membrane composed of phospholipids bilayer used to deliver drugs or genetic material into a cell. Liposomes can be composed of naturally derived phospholipids wit mixed lipid chains(like egg, phosphatidylethanolamine), or of pure components .

The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection . Liposomes can be created by sonicating phospholipids in water.

5.Gliadin Nanoparticles.

To improve bioavailability anti-H.pylori effects of antibiotics, mucoadhesivegliadin.Neutral amino acid can promote hydrogen bonding interaction with the mucosa whereas the lipophilic components can interact within biological tissue by hydrophilic interaction.

6.Polymeric Nanoparticles.

Polymeric Nanoparticles have been invented by Speiser et al. They represent interesting alternative as drug delivery systems to liposomes. They usually exhibit a long shelf life & a good stability on storage. Nanoparticles can be prepared either from preformed polymers, such as polyesters (i.e.polylactic acid), or from a monometer during its polymerization, as in the case of alkylcyanoacrylates.

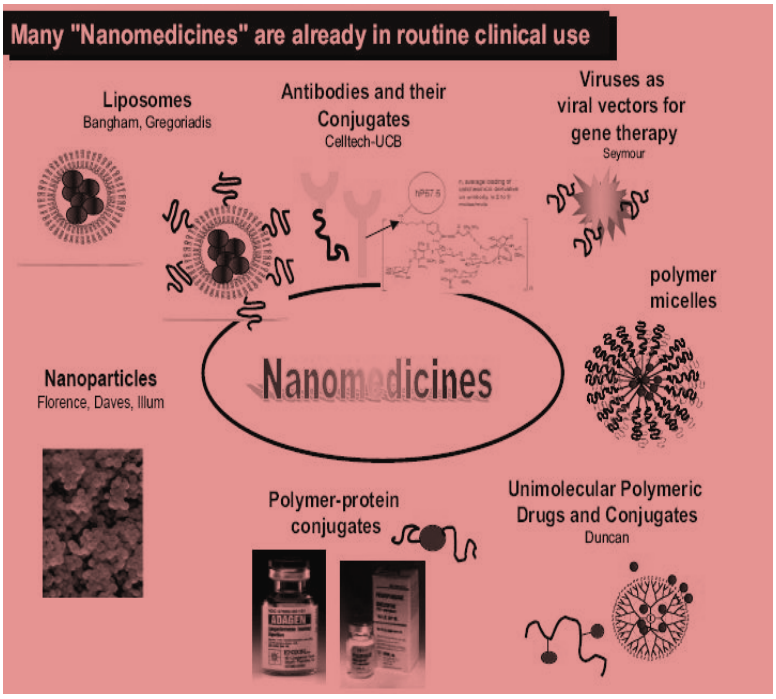


Figure 1.5: Various Nanomedicine

7.Solid lipid Nanoparticles.

Solid Lipid Nanoparticles have been developed as alternative delivery system to conventional polymeric nanoparticles. SLNs are sub-micron colloidal carriers.

Advantages.

- ❖ Avoidance of coalescence leads to enhanced physical stability
- ❖ Reduced mobility of incorporated drug molecules leads of drug leakage
- ❖ Static interface solid/liquid facilitates surface modification.

8.Others

Gold nanoparticles stabilized by thiol functionally are extraordinarily stable. A common synthesis involves the reduction of a gold salt in the presence of capping agent molecules such as thiols, citrates or phosphines. The synthesis of gold nanoparticles with a polymer-thiol monolayer involves the mechanism of particle formation in the presence of bulky ligands.

Gold nanoparticles are very good at scattering and absorbing light. It doesn't stick as well to noncancerous cell. The results can be seen with a simple microscope. In the study, researchers found that the gold nanoparticles have 600 percent greater affinity

Advantages of Nanoparticles

- Smaller dosage form (i.e., smaller tablet)
- Decreased toxicity
- Stable dosage forms of drugs which are either unstable or have unacceptable low bioavailability in non-nanoparticulate dosage forms.
- Increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body. Faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity.
- Reduction in fed/fasted variability.
- They are suitable for different routes of administration
- Carrying capacity of nanoparticles is high
- Shelf-stability of drug increases
- Ability to sustain and control drug release patterns
- Suitable for combination therapy where two or more drug can be co-deliverd
- Both hydrophobic and hydrophilic drug can be incorporated

- System increases the bioavailability of drugs
- Imaging studies can be done by utilizing them
- It is used for targeted drug delivery of drugs
- Development of new medicines
- Toxicity and adverse drug interactions are reduced to a possible extent

E.g. polymethacrylic nanoparticles for targeting anticancer drug of doxorubicin to reduce liver toxicity.Nanoparticles posses' better stability as compared to leptosomes which make it more important for many modes of targeting.

Nanoparticles formulated as amorphous offer solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence the bioavailability.

- The methods of preparation are simple, easier and reproducible.
- A high degree of patient compliance can be achieved.
- Wide range of polymer can be used depends on the nature of the drug and usage i.e. biodegradable polymer for shorter periods, and non-biodegradable for longer periods.
- It can easily pass through syringe needle and exhibit good rheological properties.

Disadvantages of Nanoparticles.*(Bshsagar., et al., 2010)*

- The manufacturing costs of nanoparticle are high which result in overall product cost
- Solvents are toxic in nature which is used in the preparation process
- Can start immune response and allergic reactions in body
- Extensive use of poly (vinyl alcohol) as stabilizer may toxicity issues
- According to a discovery, silver nanoparticles used in socks to fight foot odor if released in water can prove detrimental to the purity of water.

- Silver nanoparticles are bacteriostatic, by which we mean that they limit the growth of bacteria. This may result in the destruction of bacteria that help in breaking down the organic matter in water treatment plants.
- The process of manufacturing nanomaterials results in the release of certain waste products. This waste can float in air or even penetrate animal and plant cells.
- Nanoparticles have large surfaces. This makes them susceptible to get absorbed by macromolecules in an animal body. They can hinder biological processes, thus intervening the functioning of nature.
- "Oral delivery via tablets or capsules is largely inefficient due to exposure of the pharmaceutical agent to the metabolic processes of the body. Therefore, a larger than necessary dose is often required and the maximum effectiveness of the drug is limited.
- Traditional intravenous (IV) administration is much more problematic. Specificity for IV injectable drugs is often low, necessitating large amounts of a drug be injected into a patient, creating a high concentration of the drug in the blood stream that could potentially lead to toxic side effects".

Limitations of Nanoparticle.*(Randy P. Carney., et al., 2008)*

For example

- Their small size and large surface area can lead to particles aggregation, making physical handling of nanoparticles difficult in liquid and drug formulations.
- In addition, small particle size and large surface area readily result in limited drug in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

Factors affecting the release of drugs from particulate carriers.

(Soppimathk s., et al., 2001)

Drug.

- position of the particle
- molecular weight
- physicochemical properties
- drug-carrier interaction
- diffusion ; desorption from the surface Particles
- type and amount of matrix material
- size and density of the particle
- capsular(or) monolithic
- extent and nature of any cross linking ; denaturation of polymerization ,presence of adjutants
- surface erosion; particle diffusion and leaching
- total disintegration of particles

Environment.

- hydrogen ion concentration
- polarity
- ionic strength
- presence of enzymes
- temperature.

Applications of Nanoparticles.*(Rakesh P. Patel., et al., 2008)*

Table 1.2: Applications of Nanoparticles

Nanomedicnes	Nanodrugs, medical devices, tissue engineering, etc.
Materials	Nanoparticles, carbon nanotubes, biopolymers, paints, coating
Chemicals and cosmetics	Nanoscale chemicals and compounds, paints, coating, etc.
Food science	Processing, nutracetical food, nanocapsules
Envirnoment and energy	Water and air purification filters, fuel cells, photovoltics
Military and security	Sensers, wepons, sensory enhancement
Electronics	Semiconductor chips, memory storage, photonics, optoelectronics
Scientific tools	Atomic force, microscopes and scanning tunneling microscope
Agriculture	Pesticides, food production

Therapeutic applications of Nanoparticles*(Shoba Rani.,2008)*

- For intracellular targeting of anti-infective drugs to combat the 'difficult to treat' intracellular infections of the human body
- For targeting of cytostatic drugs to reduce toxicity & increase therapeutic activity

- For specific targeting of anti-inflammatory drugs to areas of inflammation, by which the side effects of these drugs can be minimized
- For ocular delivery systems, to deliver pilocarpine and other miotic drugs
- As carriers for radio nucleotides for diagnostic purpose in nuclear medicine
- To improve the solubility and bioavailability of poorly soluble drugs
- For skin and hair care in the form of solid lipid nanoparticles wherein the oily core contains a wide variety of different cosmetic oils and lipophilic agents
- To deliver drugs across the brain barrier (BBB)
- To formulate sustained release preparations
- For the controlled delivery of disinfectants or algaecides into large bodies of water such as insect pest feed on colloidal particles
- For targeted delivery of proteins and peptides.

1.2.3. Polymers employed for Nanoparticles .(*Deepak Thassu .,et al., 2007)*

Synthetic polymers.

- Polylactide co glycolic acid
- Poly lactide
- Polycaprolactone
- Polymethyl methacrylate
- Poly methyl methacrylate copolymer
- Poly isobutylcyanoacrylate
- Polyhexylcyanoacrylate
- Ethyl cellulose
- EudragitRL
- EudragitRS

Natural polymers.

- Gelatin
- Lecithin
- Albumin
- Chitosan
- Casein

i. Natural biodegradable polymers used to prepare nanoparticles alginates

Alginates are linear, unbranched polysaccharides composed of randomchains of Guluronic and mannuronic acids. In aqueous media, the sodium ions from salts of these anionic, heteropolymers exchange with divalent cations, such as calcium, to form water-insoluble gels. Because of the favorable conditions during manufacture, alginates are ideal carriers for oligonucleotides, peptides, proteins, Water-soluble drugs or drugs that degrade in organic solvents. Alginates are non immunogenic and available in a wide range of molecular weights as characterized by their inherent viscosity. Alginate nanoparticles are prepared by extruding an aqueous sodium alginate solution through a narrow-bore needle into an aqueous solution of a cationic agent, such as calcium ions, chitosan, or poly-l-lysine. These cations cross-link the Guluronic and mannuronic acids to form an egg-box structure That forms the core of the gel matrix. *In vivo*, therapeutic agents are released whether matrix redissolves due to the reversible exchange of divalent cations with monovalentions, especially sodium present in physiological fluid.

Chitosan

Chitosan is a natural polymer obtained by deacetylation of chitin, a component of crab shells. It is a cationic polysaccharide composed of linear β (1, 4)-linked d-glucosamine.the various methods used to prepare chitosan-based nanoparticles and their applications has been

extensively reviewed. Chitosan can entrap drugs by numerous mechanisms including chemical cross-linking, ionic cross-linking, and Ionic complexation.

Gelatin

Gelatin is a natural, biodegradable protein obtained by acid- or base-catalyzed Hydrolysis of collagen. It is a heterogeneous mixture of single- or multi-stranded Polypeptides composed predominantly of glycine, proline, and hydroxyproline Residues and is degraded in vivo to amino acids. Gelatin nanoparticles are prepared by a two-step, de solvation process. The concentrated gelatin liquid particles are isolated and hardened by chemical cross-linking with glutaraldehyde. Alternately, these particles can be prepared using a simple o/w emulsion or w/o/w micro emulsion method. Gelatin nanoparticles have been used to deliver paclitaxel, methotrexate, doxorubicin, DNA, double-stranded oligonucleotides, and genes. Pegylation of the particles significantly enhances their circulation time in the blood stream and increases their uptake into cells by endocytosis. Antibody-modified gelatin nanoparticles have been used for targeted uptake by lymphocytes.

Pullulan

Similar to dextran and cellulose, the glucans in Pullulan are water-soluble, linear Polysaccharides that consist of three α-1, 4-linked glucose molecules polymerized By α-1,6 linkages on the terminal glucose . Pullulan is a fermentation product of the yeast aureobasidiumpullulans. Pullulan nanoparticles have been prepared by Dialysis of an organic solution against water. In one method, a reverse micellar Solution of the anionic surfactant, aerosol, in n-hexane was prepared and an aqueous solution of the drug and Pullulan added. The nanoparticles are stabilized by cross-linking with glutaraldehyde. These delivery systems have been used in delivering cytotoxic drugs, genes, and as ph-sensitive delivery systems

Gliadin

Gliadin is a glycoprotein that, as a component of gluten, is extracted from gluten rich food such as wheat flour. They are slightly hydrophobic and polar. Bioactive molecules of variable polarity can be encapsulated into gliadin nanoparticles. Gliadin nanoparticles can be prepared by a desolvation method that exploits the insolubility of this polymer in water. Briefly, gliadin nanoparticles are precipitated when an ethanolic solution of gliadin is poured into an aqueous solution. Gliadin nanoparticles have been used to deliver trans-retinoic acid, α-tocopherol, and vitamin e. Lectins have been conjugated to the surface of gliadin nanoparticles to target the colon and treat helicobacter pylori infections .

ii. Synthetic biodegradable polymers used to prepare Nanoparticles

Polylactide and polylactide-co-glycolide

The hydrophobic PLA may be used alone or copolymerized with poly-glycolic acid to form a range of PLGA of widely varying polymeric ratios and hence physicochemical properties. These FDA-approved polymers have been widely used in drug delivery including nanoparticles. PLA and PLGA polymers degrade by random Bulk hydrolysis that is catalyzed in acidic media.

Polyanhydrides

Polyanhydrides are biodegradable polymers with a hydrophobic backbone and a hydrolytically labile anhydride linkage. They are synthesized by ring-opening Polymerization and degrade by surface hydrolysis. The application of Polyanhydrides has been limited to film and microsphere formulation for sustained release of a drug or protein at the site.

Poly-ϵ-caprolactones

Methods used to prepare nanoparticles using poly-ε-caprolactones have been previously reviewed and include emulsion polymerization, solvent displacement, Dialysis, and interfacial polymer deposition. These semi crystalline polymers are chemically stable,

possess a low glass transition temperature, and degrade slowly. Poly-ε-caprolactone Nanoparticles have been used as vehicles to deliver a wide range of drugs including tamoxifen, retinoic acid, and griseofulvin.

Polyalkyl-cyanoacrylates

Polyalkyl-cyanoacrylate nanoparticles are prepared by the conventional Emulsion-evaporation technique. In addition to sustaining drug release, PACA Nanoparticles have the ability to overcome multidrug resistance at both the cellular and sub cellular levels. The potential for targeted delivery of PACA nanoparticles to cells has been demonstrated by conjugation of polysaccharides to the surface

Non biodegradable polymers used to prepare Nanoparticles

Polymethacrylate and Polymethyl methacrylate have been widely used in a variety of pharmaceutical and medical applications Incorporation of poly-acrylic acid into nanoparticles increased the transfection efficiency of DNA The side chain of PMMA can be modified to make these polymers possess ph-dependent solubility and has been used to prepare pH-sensitive Nanoparticles to increase the oral bioavailability.

1.2.4. Preparation techniques of Nanoparticles*(Vyas S.P andKhar R.K., 2002)*

The selection of the appropriate method for the preparation of nanoparticles depends on the physicochemical characteristics of the polymer and the drug to be loaded on the contrary; the preparation techniques largely determine the inner structure, *in-vitro* release profile and the biological fate of these polymeric delivery systems. Two types of systems with different systems with different inner structures are apparently possible.

- A matrix type of system consisting of an entanglement of oligomer or polymer units(nanoparticles/nanospheres)
- A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell(nanocapsules).

Structure of nanospheres and nanocapsules.

These methodologies are conveniently classified as follows.

1. Amphiphillic macromolecule cross linking
    a. Heat cross linking
    b. Chemical cross linking

Polymerization based methods

    a. Polymerization of monomers *in situ*
    b. Emulsion(micellar)polymerization
    c. Dispersion polymerization
    d. Interfacial condensation polymerization
    e. Interfacial complexation.

2. Polymer precipitation methods
    a. Solvent extraction/evaporation
    b. Solvent displacement(nanoprecpitation)
    c. Salting out.

Several methods exist for the preparation of nanoparticles. When synthetic polymers are used, they are typically dissolved in a convenient solvent followed by precipitation in a liquid environment leading to nanoparticle formation. The drug intended to be encapsulated in the particles is usually incorporated in the process during the polymer solvation and precipitation. Emulsification/solvent diffusion, emulsification/solvent evaporation, nanoprecpitation and salting-out methods are widely applied techniques and they have been discussed in several reviews.

Polymerization method *(Mohanraj VJ and chen Y., 2006)*

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by

adsorption onto the nanoparticles after polymerization completed.This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles Nanocapsules formation and their particle size depends on the concentration of the surfactants and stabilizers used.

Emulsion-solvent evaporation *(Lamprecht Alf., 2009)*

Polymer and drug are dissolved in a suitable volatile solvent which is Immiscible with water. This solution is emulsified in an aqueous solution Containing stabilizer by conventional emulsification techniques. Droplet size can be further reduced by using a high-energy source. Continuous emulsification under mixing prevents coalescence of organic droplets and allows the spontaneous evaporation of the solvent at room temperature and the formation of the colloidal particles. Following evaporation of organic phase under reduced pressure or vacuum produces a fine aqueous dispersion of nanoparticles. This technique was principally applied to the preparation of particles from water insoluble polymers.

Solvent-displacement, -diffusion, or Nanoprecpitation*(Beck-Broichsitter M., et al.,2010)*

A solution of polymer, drug and lipophilic stabilizer (surfactant) in a semi polar solvent miscible with water is injected into an aqueous solution (being a non-solvent or anti solvent for drug and polymer containing another stabilizer under moderate stirring. Nanoparticles are formed instantaneously by rapid solvent diffusion and the organic Solvent is removed under reduced pressure. The Velocity of solvent removal and thus nuclei formation is the key to obtain particles in the nanometer range instead of larger lumps or agglomerates.

Salting-out

Although a less common method of preparation, by adding a solution of polymer and drug in a water miscible solvent to an aqueous solution containing a salting -out agent and a stabilizer under stirring, small droplets can be obtained. Dilution of the resulting *o/w*

emulsion with water forces diffusion of organic solvent into the aqueous phase. The remaining polymer together with the drug produces particles in the nano-size range. The resulting dispersion often requires a purification step to remove the salting-out agent.

In emulsification/solvent diffusion *(Randy.et al.,2008)*

Nanoparticles are formed when the saturation limit of a partially water-miscible solvent (Benzyl alcohol) is exceeded by addition of water. The phase separation is accompanied by vigorous stirring. The separated solvent is removed by cross-flow filtration.

Purification techniques of Nanoparticles.*(Langmuir.,2010)*

Nanoparticles are intended to be used as pharmaceutical dosage forms in humans.

Three important processes are performed.

- Purification
- Freeze-drying
- Sterilization

Purification of Nanoparticles.

In this method, various toxic impurities such as organic solvents, electrolytes, surfactants, stabilizers and large polymer aggregates can be found. Simple filtration will only remove polymer aggregates, while other impurities require sophisticated procedures.

Most common procedures.

- Gel filtration.
- Dialysis.
- Ultracentrifugation.

These methods are not satisfactory because they are restricted to the lab scale or incapable of eliminating molecules with high molecular weight.

Cross-flow filtration method.

In this method, the nanoparticle suspension is filtered through membranes, with the direction of the fluid being tangential to the surface of the membranes.

Freeze-drying of Nanoparicles.

It is one of the method used to ensure the long-term conversation of polymeric nanoparticles

Method.

This technique involves the freezing of the suspension and the subsequent elimination of its water content by sublimation under reduced pressure. After complete desiccate, nanoparticles are obtained in the form of dry, free-flowing powder that is easy to handle and store.

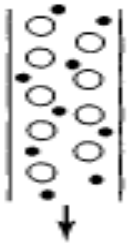
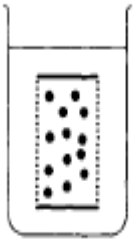
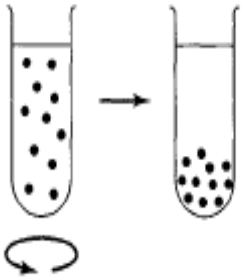
Sterilization of Nanoparticles

Some of the well-established methods of sterilization such as filtration 0.22µm filters are not adequate for nanoparticle suspensions because microorganisms and nanoparticles are generally similar in size (0.3-1µm). Therefore, this method is best achieved by using aseptic techniques throughout their processing and preparation.

Autoclaving (most heat sterilization) and Ɣ-irradiation are the techniques that can be applied for terminal sterilization. This technique may have impact on the physicochemical properties of the particles.

1.2.5.Main methods for the purification of Nanoparticles.*(Mathlowitz Edith., 2009)*

Table 1.3: Main methods for the purification of nanoparticles on the laboratory scale

Method	Schematic Principle	Drawback
Gel filtration		Removal of high molecular weight impurities difficult
Dialysis		Removal of high molecular weight
Ultracentrifugation		impurities difficult

Various approaches to maintain the mechanism *(Dong M. Shin., et al., 2007)*

Passive and active targeting.

- Passive targeting approaches
- Active targeting approaches.

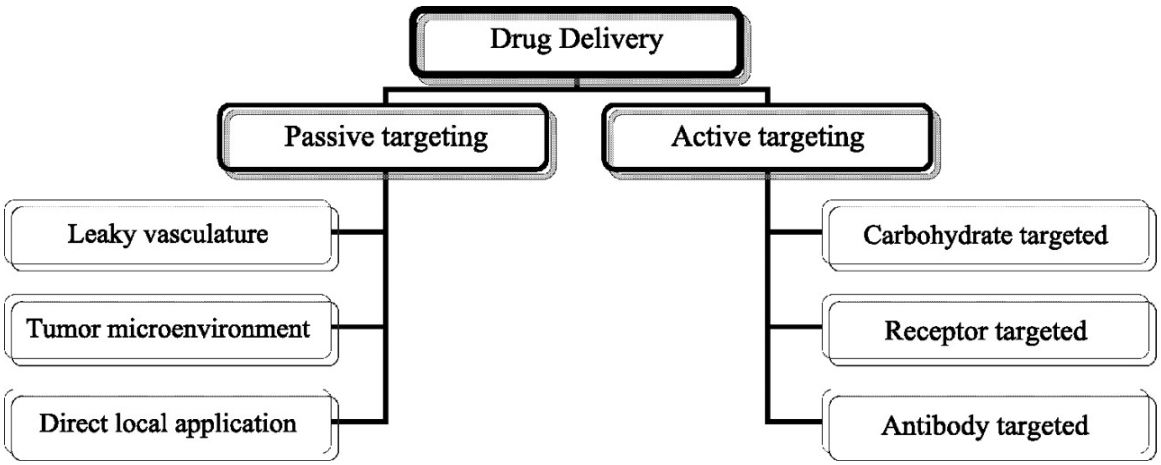


Figure 1.6: various approaches to maintain the mechanism

Passive targeting approaches.

Takes advantage of natural anatomical structures or physiological processes, which direct carrier in vivo distribution.

- ❖ Pathophysiological factors – Inflammation, Infection, EPR effect
- ❖ Physicochemical factors – Size, Molecular weight
- ❖ Anatomical opportunities – Catheterization, Direct injection
- ❖ Chemical approaches – Prodrugs, Chemical delivery systems.

Active targeting approaches

Carrier specificity can be enhanced, through surface functionalization with site-directed ligands which bind or interact with specific tissues

- ❖ Biochemical targets – Organs, Cellular, Organelles, Intracellular
- ❖ Physical/External Stimuli – Ultrasound, Magnetic field
- ❖ Pretargeting/Sandwich targeting
- ❖ Promoter/Transcriptional targeting.

Marketed products of novel formulations

Table 1.4: Marketed products of novel formulations

Company	**Trade name**	**Composition**	**Indication**	**Administration**
Enzon	Abelect	Liposomal amphotericin B	Fungal infection	Intravenous
Berna Biotech	Epaxal	Liposomal IRIV Vaccine	Hepatitis A	Intramuscular
Novavax	Estrasorb	Micellular estradiol	Menopausal therapy	Topical
Nektar, Hoffmann-La Roche	Pegasys	PEG–a-interferon 2a	Hepatitis B, Hepatitis C	Subcutaneous
Genzyme	Pegasys	Poly(allylamine hydrochloride)	End-stage renal disease	Oral

Health implications of Nanoparticles.(*Rakesh P. Patel.,et al., 2008)*

Nanoparticles can enter the human body in several ways;

(i) The lungs where a rapid translocation through the blood stream to vital organs is possible, including crossing the BBB,

(ii) The intestinal tract,

(iii) The skin

a) Skin

Particles 500–1000 nm in size, theoretically beyond the realms of nanotechnology, can penetrate and reach the lower levels of human skin, 128 and smaller particles are

likely to move deeper into the skin particles are often used in sunscreens to absorb UV light and therefore to protect skin against sunburn or genetic damage. It has been reported by Lademann et al in that micrometer-sized particles of get through the human stratum corneum and even into some hair follicles – including their deeper parts.

b) Intestinal tract

The epithelium of the small and large intestines is in close contact with ingested material so that nutrients can be utilized. A mixture of disaccharides, peptides, fatty acids, and monoglycerides generated by digestion in small intestine are further transformed and taken in the villi.. Charged particles, such as carboxylated polystyrene nanoparticles or those composed of positively charged polymers exhibit poor oral bioavailability through electrostatic repulsion and mucus entrapment. The smaller the particle diameter the faster they could permutate the mucus to reach the colonic enterocytes; 14 nm diameter permeated within 2 min, 415 nm particles took 30 min, while 1000-nm particles were unable to translocate this barrier.

c) Lung

Based on three particle-types titanium dioxide , carbon black, and diesel particles, hazard studies in rats demonstrate that ultrafine or nanoparticles administered to the lung produce more potent adverse effects in the form of inflammation and subsequent tumors compared with larger sized particles of identical chemical composition at equivalent mass concentrations or intratracheally-instilled doses. Surface properties, such as surface chemistry and area, may play a significant role in nanoparticle particle toxicity.

1.3.Antilipedimicdrugs

Lipids.*(Anne Marie Helmenstine.,2005)*

The term 'lipid' was first used by the German biochemist Bloor in 1943 for a major class of tissue components and foodstuffs.

A lipid is a fat-soluble molecule. To put it another way, lipids are insoluble in water but soluble in at least one organic solvent. The other major classes of organic compounds (nucleic acids, proteins, and carbohydrates) are much more soluble in water than in an organic solvent. Lipids do not share a common molecule structure.

Lipids are biomolecules which are soluble in organic non-polar solvents. Consequently, fats and lipids are insoluble in water. Glycerides and waxes form a sub-group of compounds which have an ester as the major functional group and include.waxes, triglycerides, and phospholipids. Lipids without ester functional groups include.steroids, fatty acids, soaps, sphingolipids, and prostaglandins.

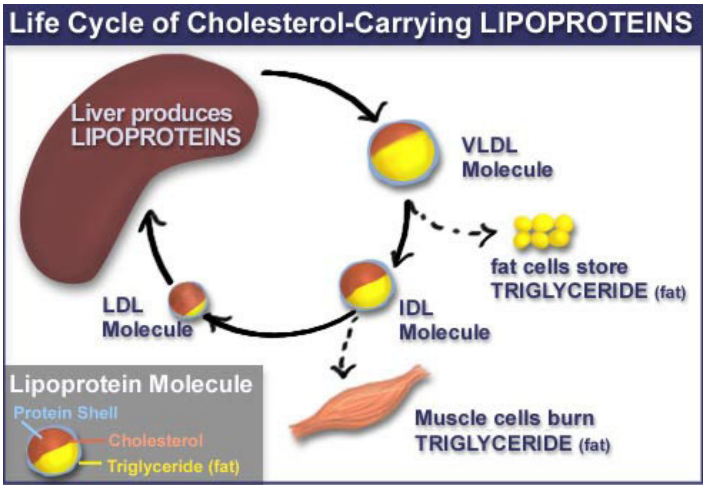


Figure 1.7: Life cycle of cholesterol

Definition of lipids(*James Richard Fromm.,1977)*

A lipid is defined as a water-insoluble biomolecule which has a high solubility in nonpolar organic solvents such as chloroform. The simplest lipids are the fats, which are triesters made up of one glycerol and three fatty acids.

The term fats is also used as a general synonym for lipids, so the more precise terms triacylglycerols or triglycerides are preferable for the simplest lipids. Triacylglycerols are used primarily for energy storage in animals. More complex lipids, the phospholipids, glycolipids, and cholesterol, are the major constituents of biological cell membranes.

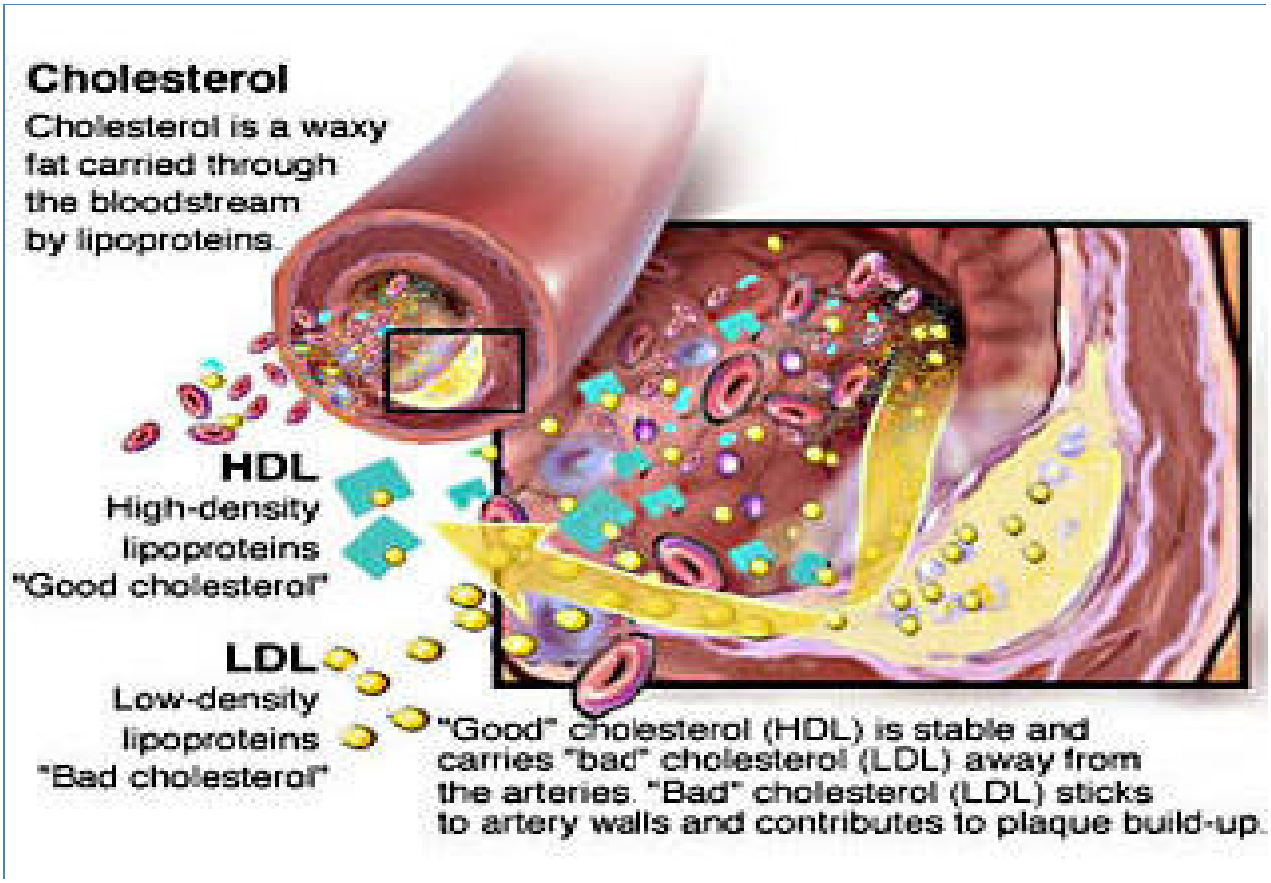


Figure 1.8: Types of lipids

Examples of common lipids*(Charles E.Ophardt.,2003)*

Examples of common lipids include

- Butter
- vegetable oil
- cholesterol and other steroids
- waxes
- phospholipids
- Fat-soluble vitamins.

Classification of lipids.*(Charles E.Ophardt.,2003)*

Table 1.5: Classification of lipids

Lipid Classification and Examples	
Fatty Acids	**Glycerides**
Saturated Fatty Acids	Steroids
Unsaturated Fatty Acids	Lipoproteins
Soap (salt of fatty acid)	Triglycerides
Prostaglandins	Phosphoglycerides
Non glyceride Lipids	
Waxes	-
Sphingolipids	-

1.3.1. Lipid structure.

Although there is no single common structure for lipids, the most commonly occurring class of lipids are triglycerides, which are fats and oils. Triglycerides have a glycerol backbone bonded to three fatty acids. If the three fatty acids are identical then the triglyceride is termed a simple triglyceride. Otherwise, the triglyceride is called a mixed triglyceride.

The second most abundant class of lipids are the phospholipids, which are found in animal and plant cell membranes. Phospholipids also contain glycerol and fatty acids, plus the contain phosphoric acid and a low-molecular-weight alcohol. Common phospholipids include lecithinsand cephalins.

The most important lipids present in blood plasma include fatty acids, triglycerides, cholesterol, phospholipids and steroid hormones.

Triglycerides, cholesterol and phospholipids.

Triglycerides are esters of fatty acids (e.g., stearic C-18 or palmitic C-16) and glycerols. Most of fatty acids are saturated, whereas nonsaturated fatty acids play an important role as prostaglandin precursors and in the process of cholesterol esterification.

Cholesterol also is a cellular membrane element and precursor of steroid hormones and biliary acids.

Phospholipids are structurally similar to triglycerides, except that one minor acid is substituted by a phosphorous group and nitrogen base.

Functions of lipids*(Charles E.Ophardt., 2003)*

- Fats and lipids are important because they serve as energy source, as well as storage for energy in the form of fat cells.
- Lipids have a major cellular function as structural components in cell membranes. These membranes in association with carbohydrates and proteins regulate the flow of water, ions, and other molecules into and out of the cells.
- Hormone steroids and prostaglandins are chemical messengers between body tissues.
- Vitamins A, D, E, and K are lipid soluble and regulate critical biological processes. Other lipids add in vitamin absorption and transportation.

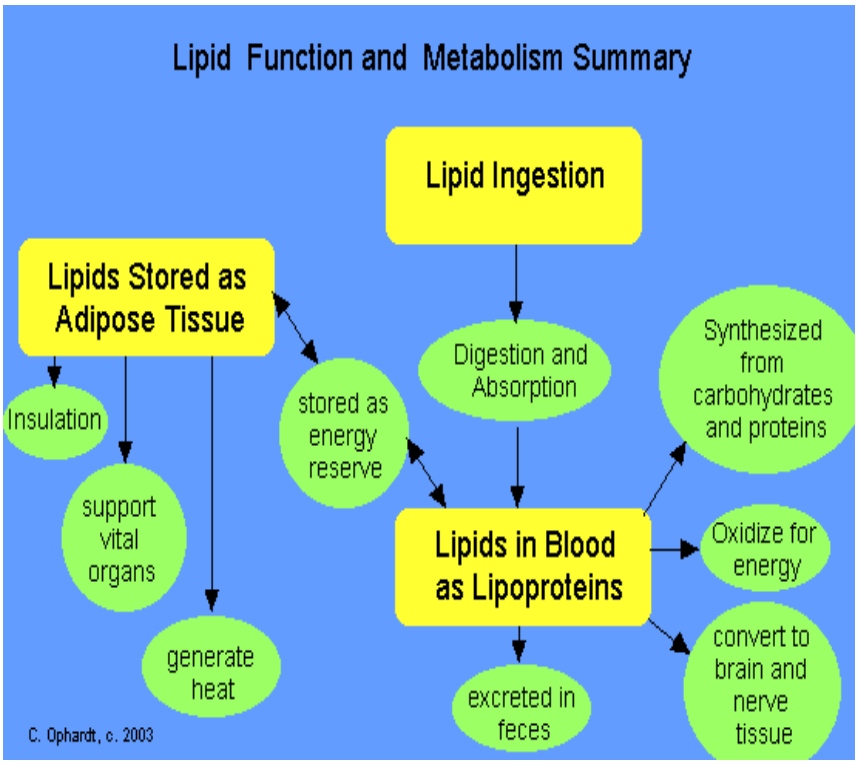


Figure 1.9: Lipid function and metabolism

Lipids act as a shock absorber to protect vital organs and insulate the body from temperature

- Energy storage, mobilization, and utilization
- Protection of organs

- Insulation
- Storage of vitamins-ADEK
- Hormone production.

Lipid metabolism impairments*(Charles E.Ophardt., 2003)*

Assessment of lipid metabolism impairments is based on plasma concentrations of cholesterol and triglycerides, and on data obtained by lipoprotein electrophoresis. Therefore, a fasting period of 14-16 hours is required before blood sampling for analysis. Disorders due to lipid metabolism impairments are associated with a high risk of atherosclerosis

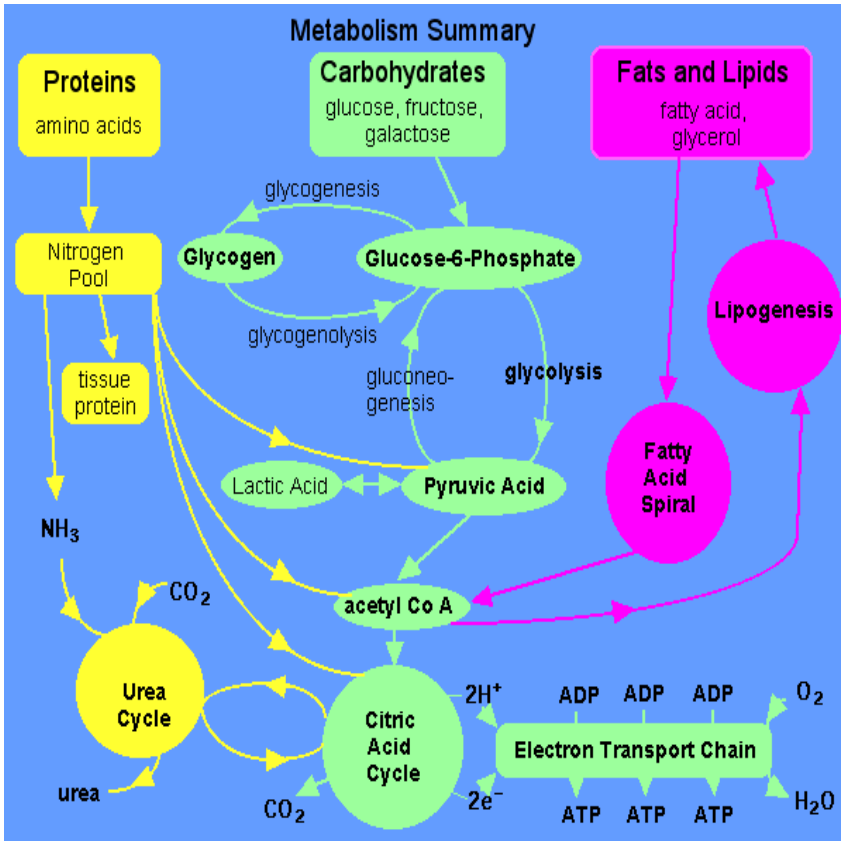


Figure 1.10: Lipid metabolism impairments

Antilipidemicdrugs. (*Tripathi K.D., 2004)*

Table 1.6: Antilipidemicdrugs

S.NO	CLASSIFICATION	NAME OF THE DRUGS
1.	HMG - CoA reductase inhibitors (Statins).	➢ Lovastatin
		➢ Simvastatin
		➢ Pravastatin
		➢ Atorvastatin
		➢ Rosuvastatin
		➢ Lovastatin
2.	Bile and sequestrants (Resins).	➢ Cholestyramine
		➢ Colestipol
3.	Active lipoprotein lipase (Fibric acid derivaties).	➢ Clofibrate
		➢ Gemfibrozil
		➢ Bezafibrate
		➢ Fenofibrate
4.	Inhibit lipolysis and triglyceride synthesis	➢ Nicotinic acid

1.4.HMG-CoA reductase inhibitors (Statins)

In 1980s, this type was the most efficacious and best tolerated hypolipidemic drugs. They competitively inhibit conversion of 3-Hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonate (rate limiting step in CH synthesis) by the enzyme HMG-CoA reductase. Therapeutic doses reduce CH synthesis by 2-50%. This results in compensatory increase in LDL receptor expression on liver cells—›increased receptor mediated uptake and

catabolism of IDL and LDL. Over long term, feedback induction of HMG-CoA reductase tends to increase CH synthesis, but a steady-state is finally attained with a dose-dependent lowering of LDL-CH levels

The daily dose for lowering LDL-CH by 30-35% is lovastatin 40mg, provastatin 40mg, simvastatin 20mg, atorvastatin 10mg, rosuvastatin 5mg. Moreover, maximum recommended doses simvastatin, atorvastatin and rosuvastatin can reduce LDL-CH by upto 45-55%, while the ceiling effect of lovastatin and pravastatin is 35-40% LDL-CH reduction. All statins produce peak V lowering after 1-2 weeks therapy.

Table 1.7: ***Mechanism of action and pattern of lipid lowering effect of important hypolipidaemic drug***(Michael E. Maragoudakis., 1970)

S.NO	CLASSIFICATION	NAME OF THE DRUGS	DAILY DOSAGE	MECHANISM OF ACTION	EFFECT ON LIPIDS (%)
1.	HMG-CoAreductaseinhibitors (Statins).	Lovastatin	(10-80mg)	▪ Decreased CH synthesis by inhibition of rate limiting HMG-CoA reductase	▪ LDL Decreased 20-55 ▪ HDL Increased 5-15 ▪ TG Decreased 10-35
		Simvastatin	(5-40mg)		
		Pravastatin			
		Atorvastatin	(10-80mg)		
		Rosuvastatin	(5-20mg)		
		Lovastatin	(10-80mg)		
2.	Bile and sequestrants(Resins).	Cholestyramine	(4-16 mg)	▪ Decreased bile acid absorption, ▪ Increased hepatic conversion of CH to bile acids ▪ Increased LDL receptors on hepatocytes	▪ LDL Decreased 15-30 ▪ HDL Increased 3-5 ▪ TG not affected, may increased in some
		Colestipol	(5-30 mg)		
3.	Active lipoprotein lipase (Fibric acid derivatives).	Clofibrate		▪ Increased Activity of lipoprotein lipase ▪ Decreased release of fatty acids from adipose tissue	▪ LDL Decreased 5-20 ▪ HDL Increased 10-20 ▪ TG Increased 20-50
		Gemfibrozil	(1200 mg)		
		Bezafibrate	(600 mg)		
		Fenofibrate	(200 mg)		

HMG-CoA reductase pathway, which is blocked by statins via inhibiting the rate limiting enzyme HMG-CoA reductase.

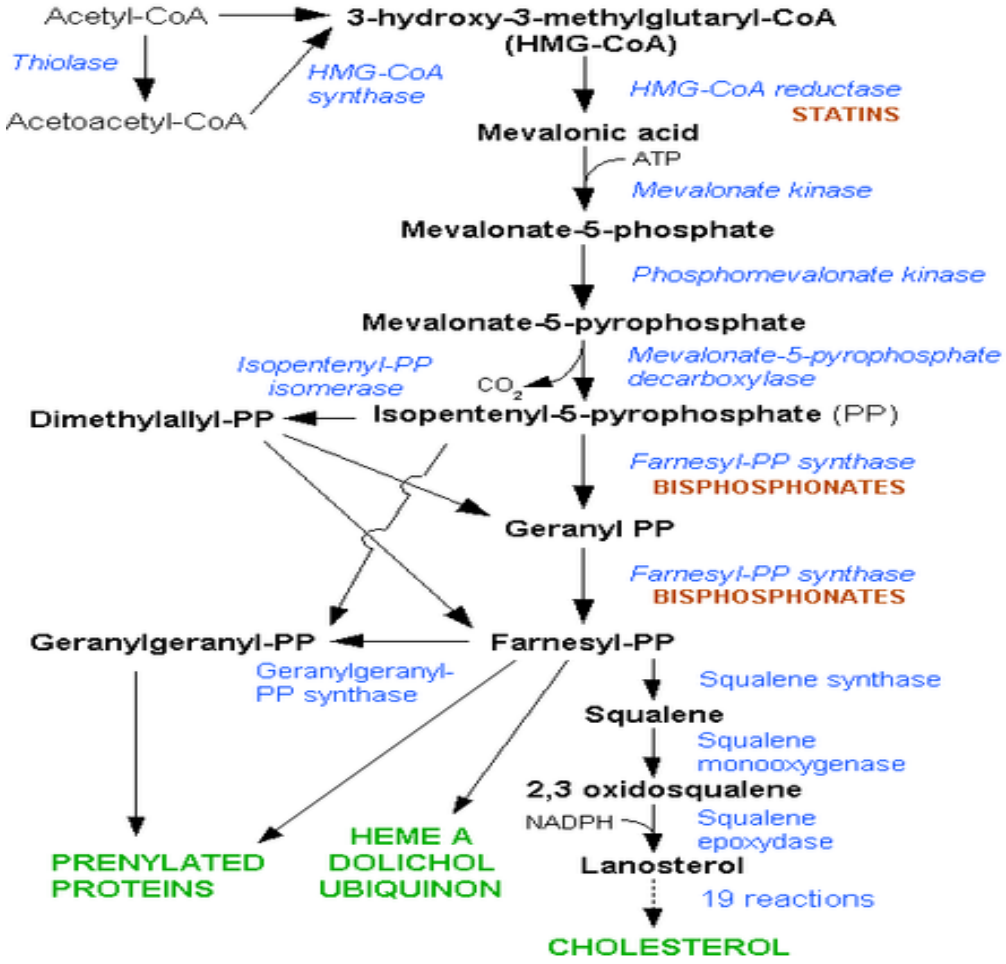


Figure 1.11:HMG-CoA reductase pathway, which is blocked by statins via inhibiting the rate limiting enzyme HMG-CoA reductase.

The more effacious statins (simvastatin, atorvastatin, rosuvastatin) given at their highe doses effectively reduce (by 25% to 35%). Because HMG-CoA reductase activity is maximum at midnight, all statins are administered at bed time to obtain maximum effectiveness. But, this is not necessary for atorvastatin and rosuvastatin, which have long plasma $t_{1/2}$

1.4.1.Other statins drugs. (*Richard N. Fogoros, M.D.,2012)*

Lovastatin.

It is the first clinically used statin; is lipophilic and given orally in the precursor lactone form.

Lovastatin 40mg

Systematic (IUPAC) name

1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate

Absorption – incomplete

Metabolism – bile

$t_{1/2}$- short (1-4 hours)

Dose – 10 to 40 mg

Simvastatin.

It is a hypolipidemic drug used to control elevated cholesterol. Simvastatin is a member of the statin class of pharmaceuticals, is a synthetic derivate of a fermentation product of Aspergillusterreus.

Systematic (IUPAC) name

(1*S*,3R,7S,8S,8aR)-8-{2-[(2*R*, 4R)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl] ethyl}-3, 7-dimethyl-1, 2,3, 7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

Absorption – incomplete

Metabolism – bile

$t_{1/2}$- 2-3 hr

Dose – 5 to 20 mg

Pravastatin.

Pravastatin (marketed as **Pravachol** or **Selektine**) is a member of the drug class of statins, used for lowering cholesterol and preventing cardiovascular disease. Initially known as CS-514, it was originally identified in a bacterium called Nocardiaautotrophica. It is also hydrophilic and given in the active form.

Pravastatin 10 mg

Systematic (IUPAC) name

(3*R*, 5R)-3,5-dihydroxy-7-((1*R*,2*S*,6*S*,8*R*,8a*R*)-6-hydroxy-2-methyl-8-{[(2*S*)-2 methylbutanoyl]oxy}-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-heptanoic acid

Absorption – incomplete

CH lowering effect - less

$t_{1/2}$- 1-3 hr

Dose – 40 to 80mg/day

Atorvastatin.

This newer statin is more potent and appears to have the highest LDL-CH lowering efficacy. All statins, including atorvastatin, prevent the production of cholesterol in the liver by blocking HMG-CoA reductase, an enzyme that makes cholesterol.

Atorvastatin 40 mg

$t_{1/2}$ - 18 - 24 hr

Dose – 10 to 40 mg

Rosuvastatin.

This is the latest and the most potent statin (10 mg rosuvastatin ≈ 20 mg atorvastatin), crestor 10 mg

$t_{1/2}$ - 18 - 24 hr

LDL-CH reduction – Greater

TG levels – raised

Dose – 5 mg

Equivalentdosages of statin groups.

Table 1.8: Equivalentdosages of statin groups

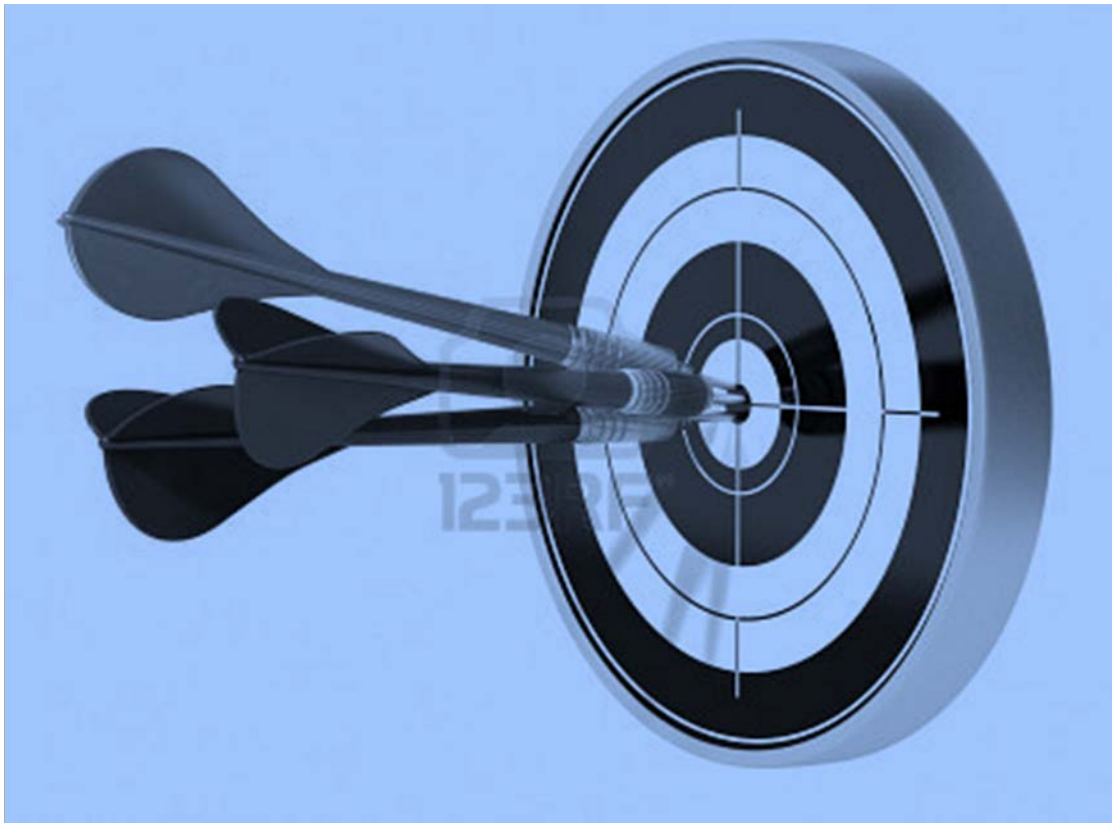
% LDL Reduction (approx.)	**Atorvastatin**	**Fluvastatin**	**Lovastatin**	**Pravastatin**	**Rosuvastatin**	**Simvastatin**
10-20%	--	20 mg	10 mg	10 mg	--	5 mg
20-30%	--	40 mg	20 mg	20 mg	--	10 mg
30-40%	10 mg	80 mg*	40 mg	40 mg	5 mg	20 mg
40-45%	20 mg	--	80 mg*	80 mg*	5–10 mg	40 mg
46-50%	40 mg	--	--	--	10–20 mg	80 mg*
50-55%	80 mg	--	--	--	20 mg	--
56-60%	--	--	--	--	40 mg	--

Adverse effects of statins.(*Tripath K.D., 2006)*

- Headache
- Nausea
- Bowel upset
- Sleep disturbances
- Rise in serum transaminase, but liver damage is rare
- Muscle tenderness
- Myopathy

Uses of statins.(*Richard N. Fogoros., 2012)*

- Statins improve blood cholesterol levels primarily by inhibiting a liver enzyme called HMG Co-A reductase.
- Reducing the size of plaques in the arteries.
- Stabilizing plaques, so they are less likely to rupture (and therefore less likely to cause acute heart attacks).
- Reducing inflammation (which is now thought to be an important component of plaque formation and rupture).
- Reducing CRP levels
- Decreasing blood clot formation (Blood clot formation at the site of plaque rupture is the cause of most heart attacks).
- Improving overall vascular function.



NEED

AND

OBJECTIVE

2. NEED AND OBJECTIVES

Application of nanotechnology in drug delivery system has opened up new areas of research in controlled release of drugs. The nanoparticle represents promising drug delivery system of controlled and targeted drug release which shows and maintains the therapeutic concentration for long period of time. The reported bio- availability of Rosuvastain is less. Hence the present study was undertaken to develop the bioavailability of the drug. While forming the nanoparticle formulation it increased the absorption and bio- availability. The present work of nanoparticles are focused on Rosuvastatin loaded gelatin nanoparticle by two step desolvation method. Hence the novel delivery system applied to antilipedimic drugs. Some biodegradable carriers degrade in the body. This problem is overcome in this nanoparticles carriers. In long term therapy fluctuations in the plasma concentrations, with high concentration peaks are common for drugs with rapid absorption and elimination. Such characteristic makes Rosuvastatin is a suitable candidate for to prepare desired nanoparticulate drug delivery system. Rosuvastatin is the widely used category of anti lipedimic drug in the treatment of high cholesterol condition.The aim of present work was to formulate by using polymer like gelatin A and Gelatin B nanoparticles containing Rosuvastatin in order to provide therapeutic effect.

2.1 Objectives

- Formulation of gelatin loaded Rosuvastatin nanoparticles by two step desolvation method.
- To characterize the nanoparticles for its physiochemical properties .
- To study the their *in-vitro* release profile and release kinetics.
- To perform Stability studies as per ICH guidelines.
- To Study the zeta potenial

PLAN OF WORK

3.PLAN OF WORK

- **LITERATURE REVIEW**
- **SELECTION OF DRUG, POLYMER**
- **EXPERIMENTAL WORK**

a) PREFORMULATION STUDIES

- **Identification of drug**
  - By FTIR spectroscopy
  - By melting point
- **Physicochemical parameters**
  - Organoleptic properties
  - Solubility profile
  - Loss on drying
- **Analytical methods**
  - Determination of λ max
  - Development of standard curve of Rosuvastatin
  - Determination of percentage purity of drug
- **Determination of compatibility for drug with polymer**
  - By DSC thermal analysis
- **Formulation of nanoparticles**
  - By two step desolvation method
- **Determination of drug loading efficiency, drug loading capacity**
- **Characterization of nanoparticles**
  - By scanning electron microscopy
- ***In-vitro* dissolution studies**
  - By dialysis bag method
- **Kinetics of drug release**
- **Stability studies**
- **Zeta potential**

LITERATURE REVIEW

4. LITERATURE REVIEW

The Literature review Indicating that advancement in nanoparticles drug delivery system is given below

Adlin jino nesalin J., *et al.*, (2009) were developed and characterized nanoparticles containing Flutamide .It is used in the treatment of prostate carcinoma having short biological half life of 5-6 hrs. It is good candidate for the formulation of sustained release dosage form. coat ratio 1:4 gives better sustained release for about 12 hrs as compared as other formulation.

Vandana singh A., *et al.*, (2010) were developed and characterized Rosiglitazone loaded gelatin nanoparticles by two step desolvation method. The sustained release of the drug could maintain the therapeutic concentration for long time. The encapsulation efficiency was found to be between in the range of 80-90%. The release profile was dependent on korsmeyer-peppas equation.

Rahul Nair K., *et al.*, (2011) was formulated and evaluated on solid lipid nanoparticles of water soluble drug Isoniazed. The entrapment efficiency of drug in the SLN has been improved. The SLNs were prepared by ethanol injection method using tristearin and phospholipon 80 H using 3^2 factorial design. The different combination of tween 80 concentrations and varied sonication time were used to prepare SLN. The prepared SLN were spherical in shape and process mean average size of 164.9 nm. It was for treatment of tuberculosis.

Tamizharasi S., *et al.*, (2009) had prepared Lamivudine loaded polymethacrylic acid nanoparticles. They were specially designed to release the drug in the vignity of target tissue. It was prepared and evaluated polymethacrylic acid nanoparticles containing lamivudine in different drug and polymer ratio by nanoprecipitation method. The particle size was found to be 121 to 403 nm.

Shanmughavel R., *et al.*, (2010) In this study preparation and characterization of biopolymeric gelatin nanoparticles for encapsulating the antimicrobial drug Sulfadiazine and its vivo drug release in phosphate buffer saline had been investicated. It was prepared by two step desolvation method. It had been proved to be effective treatment of meningococcal meningitis. The release was occurred upto 30% in a controlled manner.

DongGon Kim., *et al.*, (2006) had prepared the Retinol encapsulated chitosan nanoparticles, reconstitute in aqueous solution and application of cosmetic and pharmaceutical applications. These nanoparticles have a spherical shape and the obtained particle size is the 50 to 200nm. Solubility of Retinol is able to increase by encapsulation into chitosan nanoparticles more than 1600-fold. X-ray diffractions patterns also showed that crystal peaks of retinol were disappeared by encapsulation into chitosan nanoparticles.

Joseph Nisha Mary S., *et al.*, (2006) investigated a nanoparticles for the cancer treatment. Nanoparticles were prepared by the crosslinking method with different concentration of polymer, surfactant. So these nanoparticles showed a sustain release kinetics and good encapsulating properties.

Kalaria D.*, et al.*, (2008) were prepared Adoxorubicin loaded nanoparticles by emulsion method and stability studies was done in simulated fluids. The pharmacokinetic studies and toxicity studies were conducted in rats. So the nanoparticles showed good stability in simulated intestinal fluid and shows a sustain release, enhanced bioavailability and lower toxicity.

Kowsalya R.*, et al.*, (2005) was prepared and physicochemical evaluation of Cephalexin loaded polymethacrylic acid nanoparticles. The association of Cephalexin with nanoparticles was performed by emulsion polymerization in continuous aqueous phase. SEM indicated that Cephalexin nanoparticle have a discrete spherical structure without aggregation. The particle size, drug content and drug recovery of the nanoparticles was gradually increased with increase in the proportion of polymethacrylic acid polymer. The *in-vitro* release studies indicated that Cephalexin nanoparticles provide sustained release over a period of 21hours and follow zero order kinetics.

Lieven Baert.*, et al.*, (2009) developed a long acting formulation containing Rilivirine nanoparticles for HIV treatment. The Nanosuspensions were prepared by wet milling in aqueous carrier. The obtained particle size is 200-800nm and stable for six months, stability up to six months. Following single dose administration 3 months in dogs and 3 weeks in mice shows the sustain release

Muhammad Rafeeq P.*, et al.*, (2010) the objective of our study is to load first line anti tubercular drug, Isoniazid in chitosan nanoparticles in order to enhance bioavailability and to reduce dose frequency. Chitosan was dissolved in acetic acid aqueous solution at various concentrations, drug was dispersed in above chitosan solution kept over magnetic stirrer at room temperature for a period of 30 minute. The tripolyphosphate aqueous solution with various concentrations added drop wise to the above sonication and centrifugation with ultracentrifuge.

After freeze drying the nanoparticles were collected. SEM studies show that formulation no 2 having optimum nanosized particles. Zeta potential shows good positive potentials. It shows good encapsulation efficiency. And good release profile follows first order release kinetics

Qiang zhang., *et al.,* (1999) stearic acid nanoparticles were prepared in this study by melt-homogenization to investigate the possibility of them as a new kind of drug carrier system. Cyclosporine a as a model drug was then encapsulated into stearic acid nanoparticles. Following the establishment of assay for cyclosporine a analysis in stearic acid nanoparticles or blood samples, the encapsulation ratio of cyclosporine a to stearic acid nanoparticles was estimated and pharmacokinetics as well as bioavailability of cyclosporine a stearic acid nanoparticles after oral administration to wistar rats were studied. The mean diameter of cyclosporine a stearic acid nanoparticles was 316.1 nm, while the encapsulation ratio of cyclosporine a to stearic acid nanoparticles reached to 88.36%. The relative bioavailability of cyclosporine a stearic acid nanoparticle over reference was nearly 80%, and the time to reach maximum concentration of Cyclosporine.

YoungWook., *et al.,* (2008) was developed the natural gelatin based nanoparticles by method of modified desolvation by natural cross linker for protein drug delivery. The obtained recombinant human gelatin nanoparticles deliver a modified protein in sustained release manner without initial burst.

Yungchih kuo., *et al.,* (2005) was performing the loading efficiency of Rosuvastatin, a human immunodeficiency antiretroviral agent, on the external surfaces of polybutylcyanoacrylate and methylmethacrylatesulphopropylmethacrylate, were prepared. The experimental results indicate that the larger the polymeric nanoparticles, the smaller loading efficiency of Rosuvastatin on the two kinds of biomaterials. Freeze-drying of the two

nanoparticles, however, yields an increase in particle size and an increase in loading efficiency of Rosuvastatin, in general. Stability studies at 4°C over 6 weeks leads to an increase in particle size and a decrease in loading efficiency of Rosuvastatin. Loading efficiency of Rosuvastatin on both of the two nanoparticles decreases with a variation in pH value.

Yvette Konan N., *et al.*, (2002) had studied on preparation and characterization of sterile and freeze dried nanoparticles. By using salting out procedure to produced the sub-200nm nanoparticles. After freeze drying complete dispersion of all the types of polyester nanoparticles in presence of lyoprotectants such as saccharides and the sterility testing showed that no microbial contamination.

DRUG

AND

POLYMER PROFILE

5. DRUG AND POLYMER PROFILE

5.1: Drug profile (*McTaggart F., et al., 2001*)

CRESTOR (Rosuvastatin calcium) is a synthetic lipid lowering agent for oral administration. Rosuvastatin is an antilipidemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reducuase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Rosuvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease.

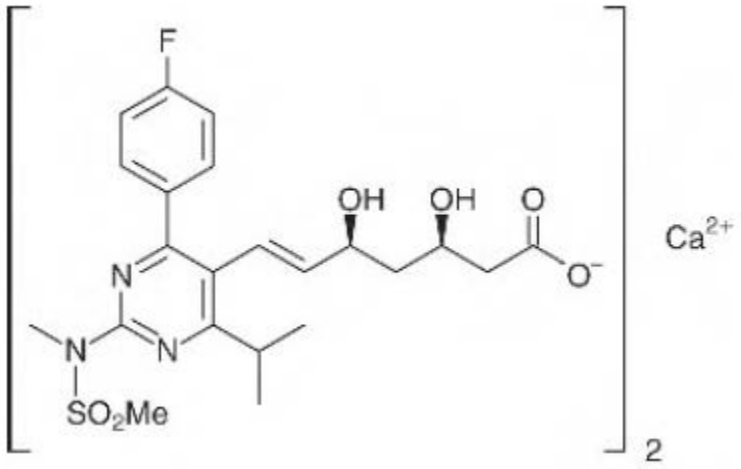
Brand name

- Astende (Lazar (Argentina))
- Cirantan (AstraZeneca (Netherlands))
- Cresadex (Drugtech (Chile))
- Crestor (AstraZeneca)
- Provisacor (AstraZeneca (Italy, Netherlands))
- Razel (Glenmark (India))
- Rosedex (Roux-Ocefa (Argentina))
- Rosimol (Sandoz (Argentina))
- Rosumed (Labomed (Chile))
- Rosustatin (Montpellier (Argentina))
- Rosuvas (Ranbaxy (India))
- Rosuvast (Bago (Argentina))
- Rosvel (Laboratorios Chile (Chile))

Chemical data

- **Molecular formula** **:** $(C_{22}H_{27}FN_3O_6S)_2Ca$
- **Molecular weight** **:** 1001.1 g/mol
- **Solubility** **:**sparingly soluble in water and methanol and slightly soluble in ethanol.
- **Melting point** **:** 155.4°C to 155°C
- **Colour** **:** Off-white to creamish white crystalline powder.
- **Plasma concentration :** reached in 3–5 hour

Molecular structure:



Category:(*McTaggart F., et al., 2001)*

- Anticholesteremic Agents
- HMG-CoA Reductase Inhibitors

Pharmacokinetics data

Bioavailability- 20%

Metabolism- Liver

Half-life- 19 h

Excretion- Urine / Faeces

Volume - 134L

Protein binding- 90%

a) Absorption:

Bioavailability is approximately 20%

b) Distribution:

Rosuvastatin is 88% bound to plasma proteins, mostly albumin. This binding is reversible and independent of plasma concentrations.

c)Volume of distribution:

134 L/kg

d) Metabolism:

Rosuvastatin is not extensively metabolized; approximately 10% of a radio labeled dose is recovered as metabolite. The metabolite is N- desmethylRosuvastatin, which is formed principally by cytochrome P450 2C9, and in vitro studies have demonstrated that N-desmethylRosuvastatin has approximately one-sixth to one-half the HMG-CoA reductase inhibitory activity of the parent compound. Overall, greater than 90% of active plasma HMG-CoA reductase inhibitory activity is accounted for by the parent compound.

e) Excretion:

Following oral administration, Rosuvastatin and its metabolites are primarily excreted in the feces (90%). After an intravenous dose, approximately 28% of total body clearance was via the renal route, and 72% by the hepatic route.

5.1.1:Mechanism of action*(Tripathy K.D., 2008)*

Rosuvastatin is a selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. First, it increases the number of hepatic LDL receptors on the cell surface to enhance uptake and catabolism of LDL. Second, Rosuvastatin inhibits hepatic synthesis of VLDL, which reduces the total number of VLDL and LDL particles.

Rosuvastatin pathway

Rosuvastatin inhibits cholesterol synthesis via the mevalonate pathway by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase is the enzyme responsible for the conversion of HMG-CoA to mevalonic acid, the rate-limiting step of cholesterol synthesis by this pathway. The active form of statins bears a chemical resemblance to the reduced HMG-CoA reaction intermediate that is formed during catalysis. Unlike Lovastatin and simvastatin, which undergo in vivo hydrolysis to their active form Rosuvastatin is synthetically produced in active form. Cholesterol biosynthesis accounts for approximately 80% of cholesterol in the body; thus, inhibiting this process can significantly lower cholesterol levels.

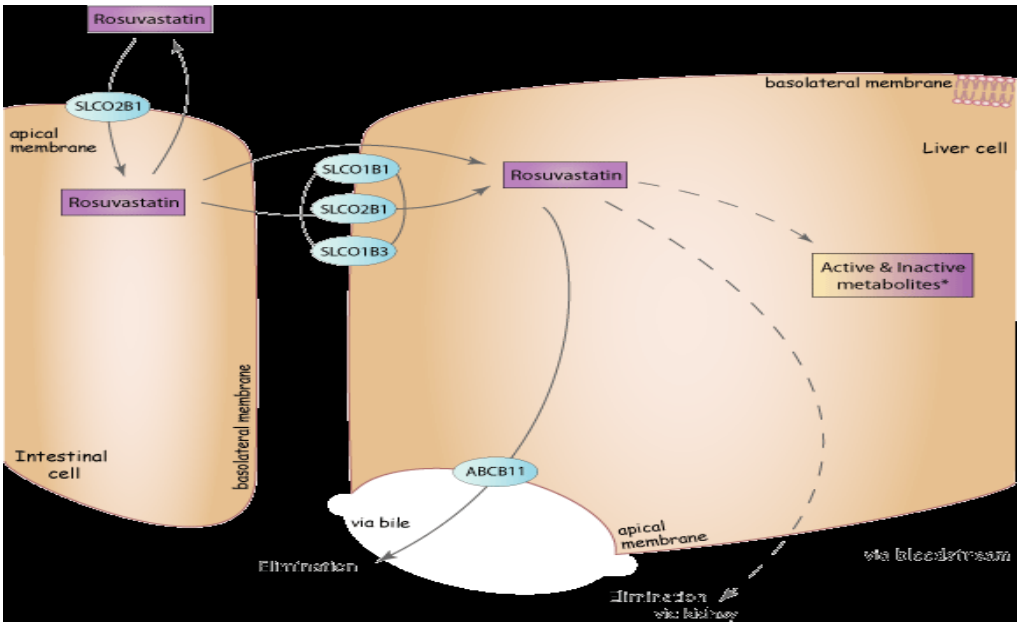


Figure 5.1: Rosuvastatin pathway

5.1.2: Adverse effects

Drug interactions ***(****Kristi Monson., et al.,2011)*

- Cyclosporine
- Gemfibrozil
- Lopinavir/Ritonavir
- Coumarin Anticoagulants
- Niacin
- Fenofibrate

Contraindications

- Hypersensitivity reactions including rash, pruritus, urticaria and angioedema
- Active liver disease
- Elevations of hepatic transaminase levels
- Pregnant women

Dosage forms

- 5 mg: Yellow, round, biconvex, coated tablets.
- 10 mg: Pink, round, biconvex, coated tablets.
- 20 mg: Pink, round, biconvex, coated tablets.
- 40 mg: Pink, oval, biconvex, coated tablets.

5.2Polymer profile*(Wayne Goates Kansas., et al., 2003)*

Gelatin is a natural, biodegradable protein obtained by acid- or base-catalyzed Hydrolysis of collagen. It is a heterogeneous mixture of single- or multi-stranded Polypeptides composed predominantly of glycine, proline, and hydroxyproline Residues and is degraded in vivo to amino acids. Gelatin nanoparticles are prepared by a two-step, de solvation process. The concentrated gelatin liquid particles are isolated and hardened by chemical cross-linking with glutaraldehyde. Alternately, these particles can be prepared using a simple o/w emulsion or w/o/w micro emulsion method. Gelatin nanoparticles have been used to deliver paclitaxel, methotrexate, doxorubicin, DNA, double-stranded oligonucleotides, and genes. Pegylation of the particles significantly enhances their circulation time in the blood stream and increases their uptake into cells by endocytosis. Antibody-modified gelatin nanoparticles have been used for targeted uptake by lymphocytes.

Description:

Gelatin occurs as a light-amber to faintly yellow-colored, vitreous, brittle solid. It is practically odorless and tasteless and is available as translucent sheets add granules.

Gelatin contasins:

- 84-90% protein
- 1-2% mineral salts
- 8-15% water
- It is free from additives and preservatives

Types of gelatin:(GMAP Publishers, 2005)

Two main types of gelatin.

Type A, is derived from collagen with exclusively acid pretreatment.

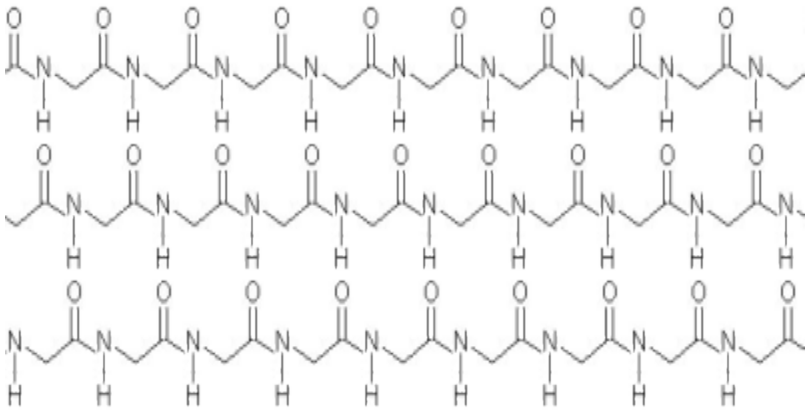
Type B, is the result of an alkaline pretreatment of the collagen.

Typical specifications for Type Aand Type B are:

Table 5.1: Typical specifications for Type Aand Type B

Parameter	**Type A**	**Type B**
pH	3.8 - 5.5	5.0 - 7.5
Isoelectric Point	7.0 - 9.0	4.7 - 6.0
Gel strength (bloom	50 – 300	50 - 300
Viscosity (mps)	15 – 75	20 - 75

Chemical formula :C102H151039N31



5.2.1:Bio physicochemical properties of gelatin

- **Color:** color varying from a very pale yellow to dark amber
- **Physical appearance:** Occurs as flakes.
- **Viscosity:**
  - 4.3-4.7 mPa s (4.3-4.7 cP) for a 6.67% w/v aqueous solution at 60°c
  - 18.5-20.5 mPa s (18.5-20.5 cP) for a 12.5% w/v aqueous solution at 60°c
- **Molecular weight:** 15000 – 250 000

5.2.2:<u>The most favorable operating conditions for the extraction of gelatin are: -</u>

Table 5.2: The extraction of gelatin

STAGES	TEMPERATURE	TIME	PH
First stage	64 ^{o}C	5 hr	7
Second stage	74 ^{o}C	3.72 hr	7
Third stage	84 ^{o}C	3.69 hr	7
Fourth stage	98 ^{o}C ,	3.83 hr	7

5.2.3: Chemical properties of gelatin:

- Together with water, it forms a semi-solid colloidgel.
- Gelatin forms a solution of high viscosity in water,
- Gelatin solutions show viscoelastic flow and streaming birefringence.
- Gelatin is put into contact with cold water, some of the material dissolves.
- solubility of the gelatin is determined by the method of manufacture.
- Gelatin is also soluble in most polar solvents.
- Gelatins are very sensitive to temperature variations.

- The viscosity of the gelatin/water mixture increases with concentration and when kept cool (≈ 4 °C).
- There are also vegetable gelatins such as agar.

Safety:

In general, when used in oral formulations gelatin may be regarded as a nontoxic & nonirritant material. Gelatin capsules adhering to the esophageal lining which may cause local irritation.

Handling precautions:

Eye protection and gloves are recommended. Gelatin should be handled in a well-ventilated environment

Uses of gelatin:*(Wayne GoatesKansas., et al., 2003)*

- binders for paper money
- cosmetics
- bonding for the tip of matches
- bakery products
- photographic film
- whipping agent in dairy products
- medicine emulsions
- hardening of jams and jellies
- treatment of wounds as a sponge

5.2.4:Pharmaceuticalaspects of gelatin: (GMAP Publishers, 2005)

P Gelatin is the most pure and perfect protein available

H It is absolutely harmless and controlled by high quality standards for such applications

A Active and readily and rapidly accepted by the body

R Reliable and safe ingredient in sensitive products

M Significant in medicinal and medical applications

A Contains practically all essential amino acids required for body cells and tissue nourishment

C Sourced from natural collagens

E Essential to all humans

U A unique and useful product used through the centuries

T Significant in thermoreversible and tabletting applications

I Truly an international product

C A clear and clean product

A Easily available from several producers who are members of gmap

L Used in several life supporting applications, such as plasma extenders.

Pharmaceutical applications: ***(****Sylvie Audoly., et al., 2006)*

Hard capsules

Hard capsules are composed mainly of gelatin, and eventually of an opacifier and coloring agents. They are obtained by dipping metallic formers called pins, into the gelatin solution, removing and allowing the solution to gel in the air and dry. Pharmaceutical customer will fill the hard capsules mainlywith powders or solid forms (tablets, …), but with the improvement of the technology including the sealing of the capsules to avoid leakage, hard capsules can also be filled with liquids or pastes when it was dedicated to soft capsules few years ago. The resistance, the elasticity and the solubility of the shell will depend on physical characteristics of the gelatin.

Soft capsules

Softgels are a one-piece, hermetically sealed capsule with an outer shell made of gelatin, water, plasticizers (glycerol, sorbitol,…) which gives elasticity and softness to the walls and sometimes coloring agents plus opacifier. Medium Bloom are preferred for elasticity and easiness to work on the machine. In this technology, the encapsulation of the drugs (oily products, non aqueous liquids, suspension and astes…) is made simultaneously with the formation of the walls. The main process used is the rotary die process.

Tablets

Gelatin is used for its binding properties. Used in direct compression or after granulation of the powder to be used in compression, gelatin or hydrolyzed collagen allow to give cohesiveness,resistance, hardness to the tablet.

Bloodplasma

Blood plasma substitute replaces blood when large volumes are lost during anaccident for example and without knowing the blood grouping. Most of the time in Europe, blood plasma substitute consists of pyrogene free water, gelatin and salts (chlorides), while in the USA starch preparation are mainly used.Blood plasma substitute reestablishes the blood pressure with the presence of relatively low molecular weight molecules. One of the advantages of plasma substitute based on gelatin is the absence of accumulation effect into the body (as it could happen with starch) due to a reasonably rapid elimination by the metabolism or excretion.

Cholostomy bags

Gelatin is used in association with other polymers as CMCor pectines and anadhesive to obtain a sticking ring allowingto fix cholostomy bags and to absorb themoisture ofthe skin. Sticking, coating and water binding properties ofgelatin are especially interesting in that case.

MATERIALS

AND

EQUIPMENT

6. MATERIALS AND EQUIPMENTS

6.1: Raw materials

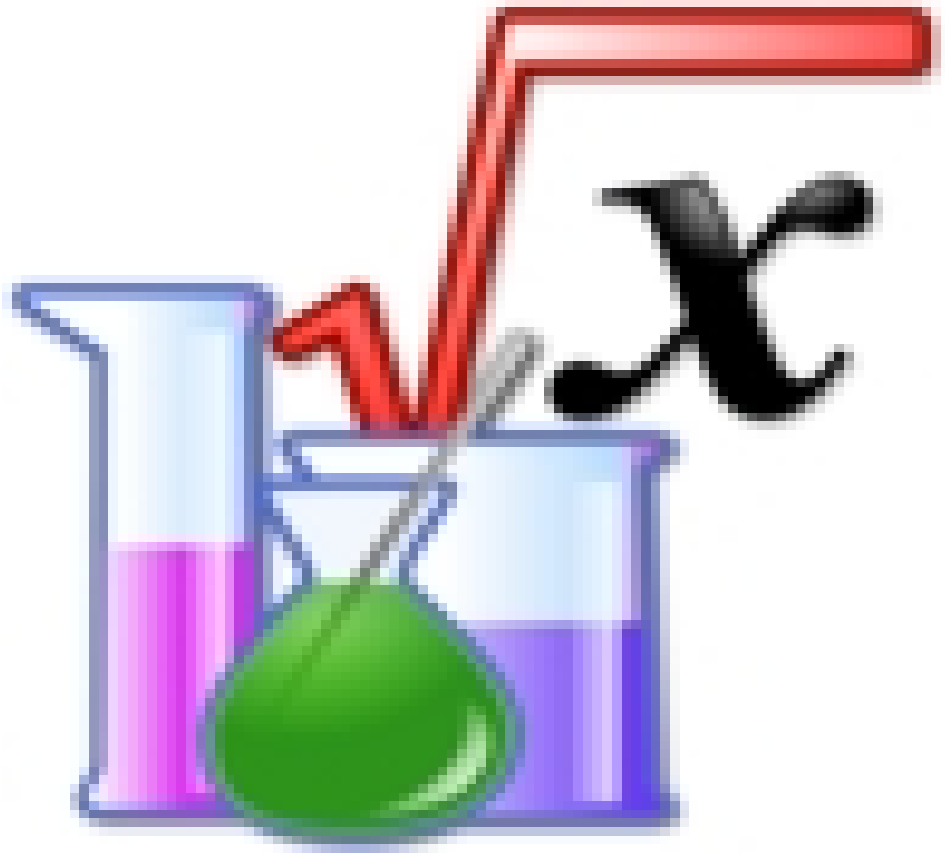
Table 6.1: Raw materials with name of the supplier

S.No	Name of raw material	Name of the supplier
1	Rosuvastatin	Torrent pharmaceuticals Ltd, Ahmedabad
2	Gelatin – A	S.D fine-chem limited, Mumbai.
3	Gelatin – B	S.D fine-chem limited, Mumbai.
4	Glutaraldehyde 25%	S.D fine-chem limited, Mumbai.
5	Distilled water	Apex, Chennai.
6	Acetone	Apex, Chennai.
7	Sodium sulfate	S.D fine-chem limited, Mumbai.
8	Sodium metabisulfite	S.D fine-chem limited, Mumbai.
9	Chloroform	S d fine-chem limited, Mumbai.
10	Methanol	Qualigens fine chemicals, Mumbai.
11	Ethanol (95%)	S d fine-chem limited, Mumbai.
12	Propylene glycol	NR chemicals, Mumbai.

6.2: Equipments

Table 6.2: Equipments with company name

S.No	Name of the Equipments	Company
1	Electronic balance	Shimadzu BL-220H
2	Sonicator	2200MH, Soltech srl, Soluzioni Tecnologirhe, Milano, italy
3	Magnetic stirrer	1-MLH, remi equipments limited, vasai
4	Freeze drying apparatus	Lyostar, S.P biotech limited.
5	Scanning electron microscope	Model-s-3400N, SEM HITACHI
6	Zeta potential	Zetasizer, Malvern, UK
7	Photon correlation spectroscopy	Malvern instruments, Germany
8	pHmeter	LI120 pHmeter, ELICO LTD
9	Humidity chamber	Labtech
10	Rotary shaker	RS-12R, remi equipments limited, vasai
11	UV spectrophotometer	Shimadzu-1700 Pharmaspec UV VISIBLE spectrophotometer
12	FTIR spectrophotometer	Shimadzu S4008
13	Differential scanning calorimeter	Shimadzu DSC 60, Japan
14	Micro ultra centrifuge	Remi equipments limited, vasai



EXPERIMENTAL WORK

7. EXPERIMENTAL WORK

7.1. Preformulation studies

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

7.1.1. Identification of drug

7.1.1.1. Identification by FTIR spectroscopy*(SkoogD.A.,et al.,1996;IP, 2007)*

Rosuvastatin discs were prepared by pressing the Rosuvastatin with potassium bromide and the spectra between 4000^{-1} to 500^{-1} cm was obtained under the operational conditions. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum in Table 8.1 and Figure 8.1

7.1.1.2. Identification by melting point *(IP, 2007)*

Melting point of the drug was determined by capillary tube method.

7.1.2. Physicochemical parameters

7.1.2.1. Organoleptic properties*(Lachman L., et al., 1991; Bankar G.S. and Rhodes C.T., 1996)*

The color, odor and taste of the drug were recorded using descriptive terminology.

7.1.2.2. Solubility study *(Merck Index, IP, 2007)*

It is important to know about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminology specified in Indian pharmacopoeia, 2007.The solubility study was shown in Table 8.2.

7.1.2.3. Loss on drying*(IP, 2007)*

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified condition. The accurately weighed 1gm of sample was transferred instoppered-glass shallow weighing bottle and accurately weighed the bottle. The bottle was transferred in oven and substance was dried at 105°C for 3 hours. The bottle was removed from oven and reweighed; loss on drying was calculated by following equation,.It was shown in Table 8.3

$$\mathbf{LOD} = \frac{\mathbf{Initialweightofsubstance} - \mathbf{Finalweightofsubstance}}{\mathbf{Initialweightofsubstance}} \times \mathbf{100}$$

7.1.3. Analytical methods

7.1.3.1. Determination of λ max (*Alka Gupta., et al., 2005)*

The absorption maximum of the standard solution was scanned between 200-400 nm regions on UV-VISIBLE spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum

Development of standard curve of Rosuvastatin in methanol: (*Alka Gupta., et al., 2005)*

25 mg of Rosuvastatin Calcium was weighed accurately and transferred into 50 ml volumetric flask and dissolved in Methanol, after dissolution the volume was made up to the mark with Methanol (500µg/ ml). Further dilution was made by pipetting 1 ml of mother liquor into 50 ml s to acquire 10 µg/ ml solution made up with methanol. The absorbance measurements of these solutions were carried out against methanol as blank at 244 nm. A calibration curve of Rosuvastatin was plotted.

Preparation of methanol solution*(IP, 2007)*

Methanol was prepared according to I.P. 2007.

Preparation of stock solution of Rosuvastatin in methanol:

Accurately weighed 100 mg of Rosuvastatin was dissolved in little quantity of 100ml of methanol and 1ml of solution was taken & make up to 10ml (100µg/ml) volume was the same to prepared standard solution having concentration of 100 µg/ ml.

Procedure:

From the stock solution, aliquots of 1, 2, 3, 4, 5,6 and 7 ml were transferred to 10 ml volumetric flasks and final volume was made to 100ml with methanol. Absorbance values of these solutions were measured against blank (methanol) at 244 nm using uv-visible spectrophotometer

Development of standard curve of Rosuvastatin in 6.8 phosphate buffer:*(IP, 2007)*

Preparation of 6.8 phosphate buffer:

Place the 50 ml of 0.2M potassium dihydrogen phosphate in a 200 ml of volumetric flask.And add specified volume of 0.2 M sodium hydroxide then add water to volume to required.

Preparation of 0.2 M sodium hydroxide:

Dissolve the sodium hydroxide in water to produce 40 to 60 %w/v solution and allow to stand and finally add 8 gm of sodium hydroxide in 1000 ml.

Preparation of 0.2 M potassium dihydrogen phosphate:

Dissolve the 27.218 gm of potassium dihydrogen phosphate in water to dilute with water 1000ml.

Preparation of stock solution of Rosuvastatin in 6.8 phosphate buffer:

Accurately weighed 100 mg of Rosuvastatin was dissolved in little quantity of 100ml of 6.8 phosphate buffer and 1ml of solution was taken & make up to 10ml (100µg/ml) volume was the same to prepared standard solution having concentration of 100 µg/ ml.

Procedure:

From the stock solution, aliquots of 1, 2, 3, 4, 5,6 and 7 ml were transferred to 10 ml volumetric flasks and final volume was made to 100ml with methanol. Absorbance values of these solutions were measured against blank (6.8 phosphate buffer) at 240 nm using uv-visible spectrophotometer

Determination of percentage purity of drug *(Dhirendra K., et al., 2010; IP, 2007)*

Standard solution Preparation:

Standard solution of Rosuvastatin calcium was prepared by dissolving 10mg of Rosuvastatin calcium in 100ml of methanol. To obtained the concentration 100µg/ml. Further diluted 5-50ml by same solvent to obtained a solution containing 10µg/ml.

Sample solution preparation:

Pure raw materials of Rosuvastatin calcium were weighed and powdered. Amount of powdered equivalent to 20 mg taken into a 200ml volumetric flask, 140 ml methanol added to dissolve the drug, cooled the flask up to the room temperature and methanol was added to make up the volume up to the mark,Centrifuged at 4000 rpm for 10mins, further diluted 5ml to 50ml with same solvent to obtain a solution containing 10 µg/ml. Same procedure was used for two other market samples of Rosuvastatin calcium.

Determination of drug-polymer compatibility (*Patil.,et al., 2009)*

The proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients to be used in the fabricating the product. Each polymer used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form.

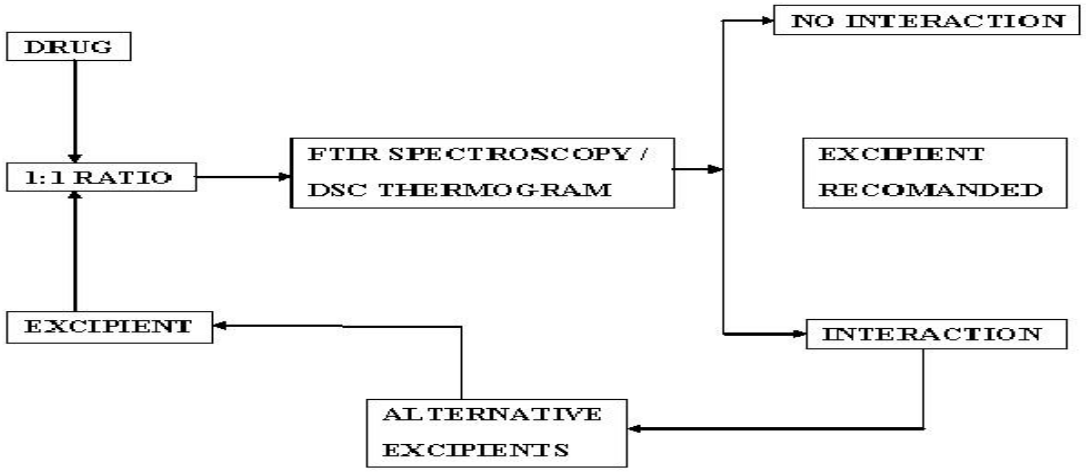


Figure 7.1: Compatibility studies

Fourier transforms infra-red (FTIR) spectroscopy *(Patil S.V., et al., 2009; IP, 2007)*

FTIR study was carried out to check compatibility of drug with polymers. Infrared spectrum of Rosuvastatin was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug with various polymers by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum .

Differential scanning calorimetry (DSC) *(Patil S.V., et al., 2009;Aulton M. E., 2002)*

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure drug, drug+ Gelatin. The study was carried out using a DSC. The 2 mg of sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-300ºc at heating rate of 10ºc /min under nitrogen flow of 30ml/min

7.2:Preparation of Nanoparticles(*Ze Lu et al.,2004)*

The nanoparticles were prepared by *in-situ*nanoemulsion polymer Two step desolvation method, by using a different drug and polymer ratios. The formulations were designated as F-1, F-2, F-3, F-4, F-5, F-6, F-7 and F-8respectively.

Table7.1: The compositions of formulations

S.NO	**Formulations**							
Ingredients (gm)	**Gelatin A ratio**				**Gelatin B ratio**			
	F1 (1:1)	**F2 (1:2)**	**F3 (1:3)**	**F4 (1:4)**	**F5 (1:1)**	**F6 (1:2)**	**F7 (1:3)**	**F8 (1:4)**
Rosuvastatin	1	1	1	1	1	1	1	1
Gelatin A	1	2	3	4	-	-	-	-
Gelatin B	-	-	-	-	1	2	3	4
Sodium sulphate (20%) ml	2	2	2	2	2	2	2	2
Sodium megabisulphate (12%) ml	5	5	5	5	5	5	5	5

Two step desolvationmethod:(*Ze Lu et al.,2004)*

Gelatin (200 mg) was dissolved in 10 ml of water containing of 2% Tween 20. The solution was heated at 40°C with constant stirring at 300 rpm. To this solution, 2 ml of a 20% aqueous solution of sodium sulfate was added slowly, followed by 1 of isopropanolcontaining2 mg of Rosuvastatin. A remaining of sodium sulfate solution (5.5–6 ml) was added untilthesolution turned turbid, (which indicated the formation of gelatin aggregates.) Approximately 1 ml of distilled water was then added until the solution turned clear. An aqueous solution of glutaraldehyde[25%, (0.4 ml)] was added to cross-link with the gelatin. Sodium metabisulfitesolution [12%, (5 ml)] was addedafter 5 minutes to stop the cross-linking process. After 1 hour, the crude product was purified on a Sephadex G-50 column. The nanoparticle containing fraction was lyophilized in a freeze drier over a 48 hour period.

7.3. Characterization of Nanoparticles

7.3.1. Characterization of Rosuvastatinloaded gelatin Nanoparticles

Encapsulation efficiency*(vandanasingh ., et al., 2010)*

The determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined(w) by spectrophotometry at 240 nm. A standard calibration curve of drug was plotted. The amount of drug in the supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively (W-w) will give the amount of drug entrapped in the pellet.

$$\text{Drug encapsulation efficiency} = \frac{\text{Amount of drug bound by total amount of nanoparticles}}{\text{Amount of drug taken.}} \times 100$$

Particle size distribution *(MaincentPandKimy., 1997)*

Every sample was diluted with phosphate buffered saline pH 6.8.The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of nanoparticles using a Malvern zeta sizer. Samples were prepared by dilution with phosphate buffer saline pH 6.8. The zeta potential value was calculated by the software using Smoluchowski'sequation.It was shown in Figure 8.15

Scanning electron microscopy (SEM)

The surface morphology (roundness, smoothness and formation of aggregate and the size of nanoparticles formulation were studied by SEM. The solid sample of freeze dried nanoparticle were dispersed in water for SEM analysis was coated with a thin layer of platinum or gold using the PVD process at a 30MA current from the distance of 50nm during

Fourier transform infra-red (FTIR) spectroscopy *(Patil S.V., et al., 2009; IP, 2007)*

FTIR study was carried out to check compatibility of drug with polymers. Infrared spectrum of Stavudine was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug with various polymers by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented in Table 8.1 and also shown in Figure 8.1

Differential scanning calorimetry (DSC)*(Patil S.V., et al., 2009)*

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure drug, drug+ Gelatin. The study was carried out using a DSC. The 2 mg of sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-300ºc at heating rate of 10ºc /min under nitrogen flow of 30ml/min.

Study of in-vitro releases*(vandanasingh .,et al.,2010)*

In vitro release study across cellophane membrane (cut off 3500 Da) precluding gelatin was performed in a specially designed diffusion chamber consisting of two compartments separated by the membrane. Drug loaded nanoparticles (5 ml equivalent to 8 mg of the drug) were placed in the donor compartment and the receptor compartment was filled with 50 ml phosphate buffer (pH 6.8). To determine the amount of Rosuvastatin diffused through the cellophane membrane, sample (1 ml) was withdrawn from the receiver compartment at the prefixed time intervals and the drug concentration was measured spectrophotometrically at 240 nm. After each withdrawal, same amount of phosphate buffer was replaced in the receiver chamberindex.

7.4. Release kinetics data *(BenoyBrataBhowmik., et al., 2006)*

To study the released kinetics of *In-vitro* drug release, data was applied to kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas.

- **Zero order**

$$C = K_0 t$$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time

t -is the time in hrs.

- **First order**

$$\mathbf{LogC = LogC_0 - Kt / 2.303}$$

Where C_0 - is the initial concentration of drug,

K - is the first order constant

t - is the time in hrs.

- **Higuchi**

$$\mathbf{Qt = Kt^{1/2}}$$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and t- is time in hrs

- **KorsmeyerPeppas**

$$\boldsymbol{Mt/ M\infty = Kt\ n}$$

Where M_t - represents amount of the released drug at time t,

M_∞- is the overall amount of the drug (whole dose) released after 12 hrs

K- is the diffusional characteristic of drug/ polymer system constant

n- is a diffusional exponent that characterizes the mechanism of release of drug.

Table 7.2: Diffusion exponent and solute release mechanism

Diffusion exponent	Overall solute diffusion mechanism
< 0.5	Quasi-Fickian diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Anomalous (non-Fickian) diffusion
1.0	Case-II transport
> 1.0	Super case-II transport

7.5: Stability studies *(Manavalan R. and Ramasamy S., 2004)*

The International Conference on Harmonization (ICH) Guidelines titled "Stability testing of New Drug Substances and Products (QIA) describes the stability test requirements for drug registration application in the European Union, Japan and the States of America

ICH specifies the length of study and storage conditions

Long-Term Testing: 25^0 C $\pm 2^0$ C at 60% RH ±5% for 12 Months

Accelerated Testing: 40^0 C $\pm 2^0$ C at 75% RH ±5% for 6 Months

In present study the selected formulation F1 was exposed up to 3 months of stability studies at room temperature, accelerated condition and refrigerator temperature ($25^0 \pm 2^0$C at 60% RH±5%RH, 40^0C $\pm 2^0$C at 750% RH ±5% RH and 5^0C $\pm 3^0$C) to find out the effect of aging on drug loading efficiency.

Procedure: *(KalariaD.R.,et al., 2008:Jospech Nisha Carry., et al., 2006)*

Stability studies were carried out by freeze dried nanoparticles as per ICH titled QIA guide lines meant for refrigerated product over a period of 90 days. Nanoparticles were kept in stability chamber with temperature at $25^0 \pm 2^0$C at 60% RH±5%RH, 40^0C $\pm 2^0$C at 75% RH ±5%RH and 5^0C $\pm 3^0$C. Sucrose (5%) was used as Cryoprotectant during the freeze drying process. Samples were withdrawn predetermined time intervals and analyzed the drug loading efficiency.

7.6. Zeta potential:*(Maincent P., et al 1997)*

The zeta potential value of the sample was measured by zetasizer.A nanoparticle formulation which gives best results between particle size, drug loading efficiency, in-vitro drug release was determined for zeta potential. Zeta potential it can help to understand the characteristics of a suspension by understanding how individual colloids interact with one another. Each colloid carries aelectrical charge is high enough, the colloids will remain discrete, disperse and in suspension. The report was showed in Figure 8.33.

RESULT

AND

DISCUSSION

8.RESULTS AND DISCUSSION

8.1: Preformulation parameters

Identification of drug

Identification by FTIR spectroscopy

The FTIRspectrum of Rosuvastatin was shown in Figure 8.1 and theinterpretations of FTIR frequencies were showed in Table 8.1

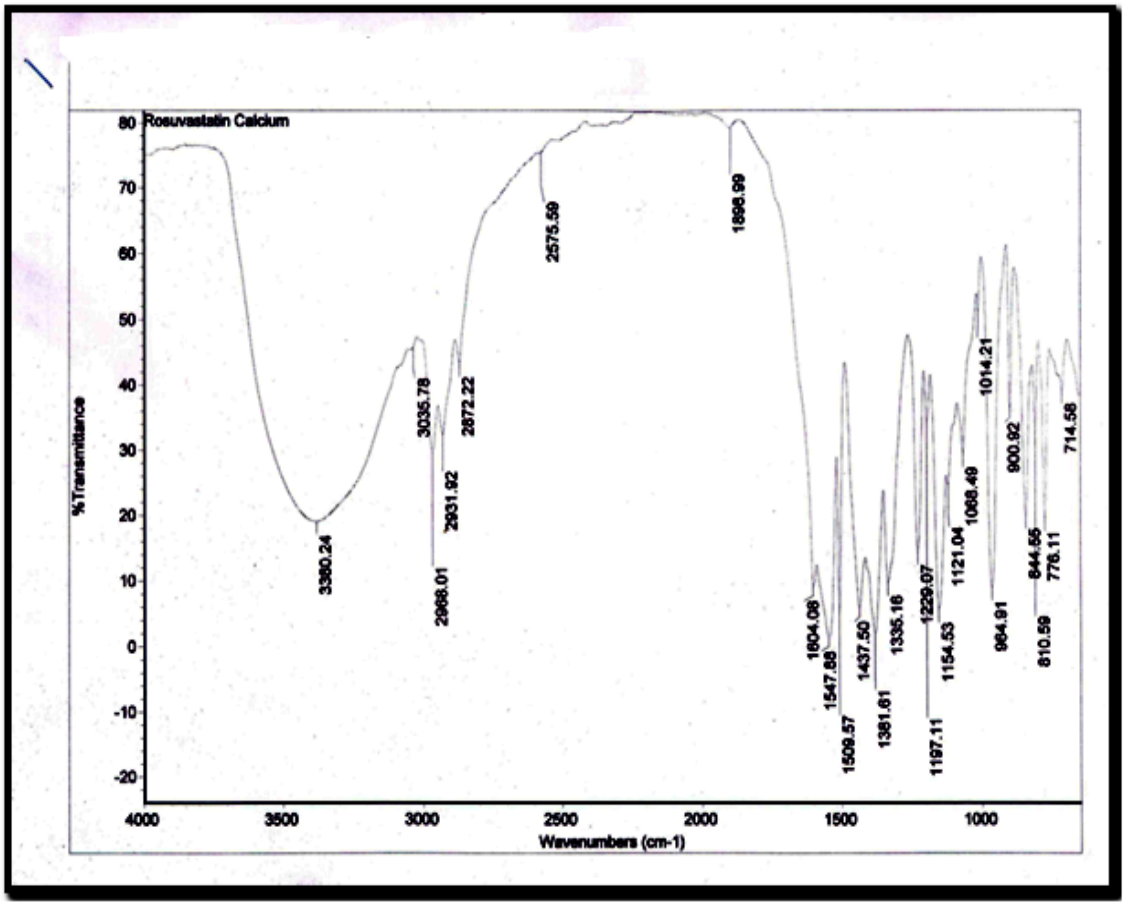


Figure 8.1:FTIR spectrum of Rosuvastatin

Table 8.1:Characteristic frequencies in FTIR spectrum of Rosuvastatin

Wave no.(cm^{-1})	Inference
1335	So_2 stretching
1068	C-F stretching
1381	C-N stretching (aromatic amine)
3035	C-H stretching
1604	C=C Skeletal stretching (Aromatic)
2968	C-H stretching
2872	CH_3 symmetric stretching
3380	C-H stretching
1728	C=O stretching
1547	CO_2 symmetric stretching
810	C-S stretching

- **Interpretation of FTIR Spectrum**

Major functional groups like Aliphatic Ethers,Aliphatic Hydrocarbons, and Primary Aliphatic Alcohols,present in Rosuvastatin showed characteristic peaks in FTIR spectrum. The major peaks were identical to functional group of Rosuvastatin. Hence, the sample was confirmed as Rosuvastatin

Melting point

Melting point values of Rosuvastatin sample was found to be in range of 155^0C to 156^0C. The reported melting point for Rosuvastatin was154.33^0C. Hence, experimental values were fitted with standard values.

Solubility study

Table 8.2: Solubilityprofile of Rosuvastatin calcium in polar and non-polar solvents

S. No	SOLVENTS	EXTENT OF SOLUBILITY	CATEGORY
1.	Distilled water	10 mg of substance was not soluble upto 100 ml of solvent	Practically insoluble
2.	Methanol	10 mg in 0.31 ml	Sparingly soluble
3.	Ethanol	10 mg in 0.36 ml	Sparingly soluble
4.	Acetonitrile	10 mg in 0.05 ml	Freely soluble
5.	Di-methylFormamide	10 mg in 0.04ml	Freely soluble
6.	Acetone	10 mg in 0.02 ml	Freely soluble
7.	Glacial acetic acid	10 mg in 0.04 ml	Freely Soluble
8.	Cyclohexane	10 mg in 0.3 ml	Soluble
9.	Chloroform	10 mg in 0.01 ml	Freely soluble
10.	Petroleum ether	10 mg in 0.45 ml	Sparingly soluble
11.	n- Butanol	10 mg in 0.07 ml	Freely soluble
12	Carbon tetra chloride	10 mg in 0.04 ml	Freely soluble
13	Benzene	10 mg in 0.09 ml	Freely insoluble
15	Iso propyl alcohol	10 mg in 0.55 ml	Sparingly soluble
16	Hexane	10 mg in 0.29 ml	Soluble
17	Diethyl ether	10 mg in 0.09 ml	Freely soluble
18	Dichloro methane	10 mg in 0.02 ml	Freely soluble

From the above data the solubility of Rosuvastatin was confirmed in various polar and non polar solvents.

Physicochemical parameters of drug

Organoleptic properties

Odour: Odourless

Colour: White or almost white

Nature: Crystalline powder.

Loss on drying

The percentage loss on drying after 3 hours was found to be as follows

Table 8.3: Percentage loss on drying for Rosuvastatin

S. No	Percentage LOD	Avg. percentage LOD
1	0.7	0.5666±0.25
2	0.4	
3	0.8	

The sample passes test for loss on drying as per the limit specified in IP, 2007 (N.M.T. 1%).It was shown in Table 8.3

Analytical methods

Determination of λ max in 0.1N methanol:

The absorption maximum for Rosuvastatin in 0.1N Methanol was found to be 244 nm and absorption maximum was shown in Figure 8.2.

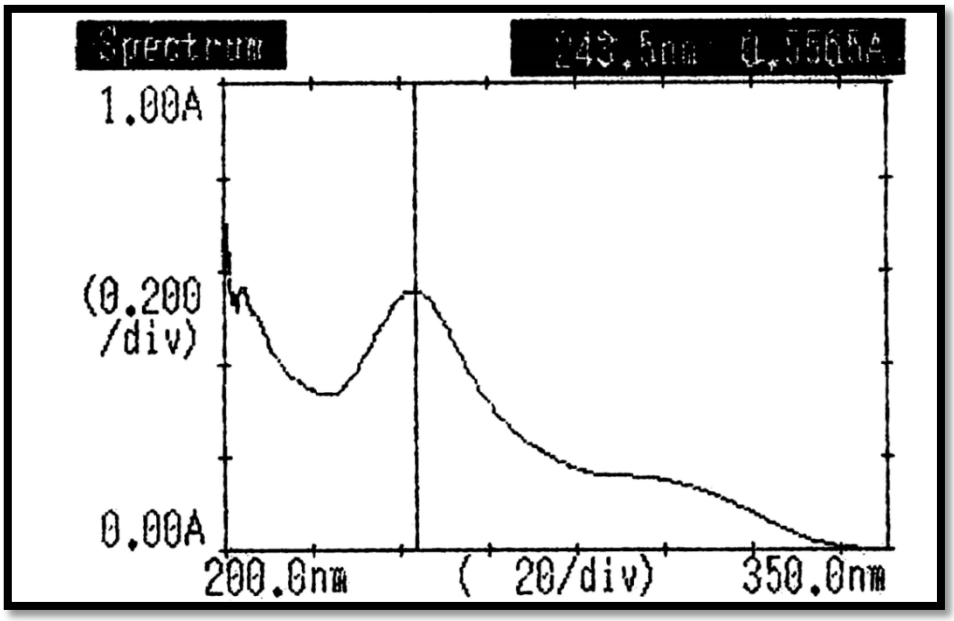


Figure 8.2: λ max observed for Rosuvastatin in 0.1N Methanol

Preparation of standard graph of Rosuvastatin in 0.1N methanol

Absorbance obtained for various concentrations of Rosuvastatinin 0.1N Methanol were given in Table.8.4.and Figure 8.3.The graph of absorbance vs concentration for Rosuvastatin was found to be linear in the concentration range of 10 µg /ml. The calibration curve parameters shown in Table.8.5So the drug obeys Beer- Lambert's law in the range of 10 µg /ml.

Table 8.4: Data of concentration and absorbance for Rosuvastatin in 0.1N Methanol

S.NO	Concentration (µg/ml)	Absorbance at 244nm
1	0	0
2	1	0.062
3	2	0.122
4	3	0.183
5	4	0.245
6	5	0.308
7	6	0.371

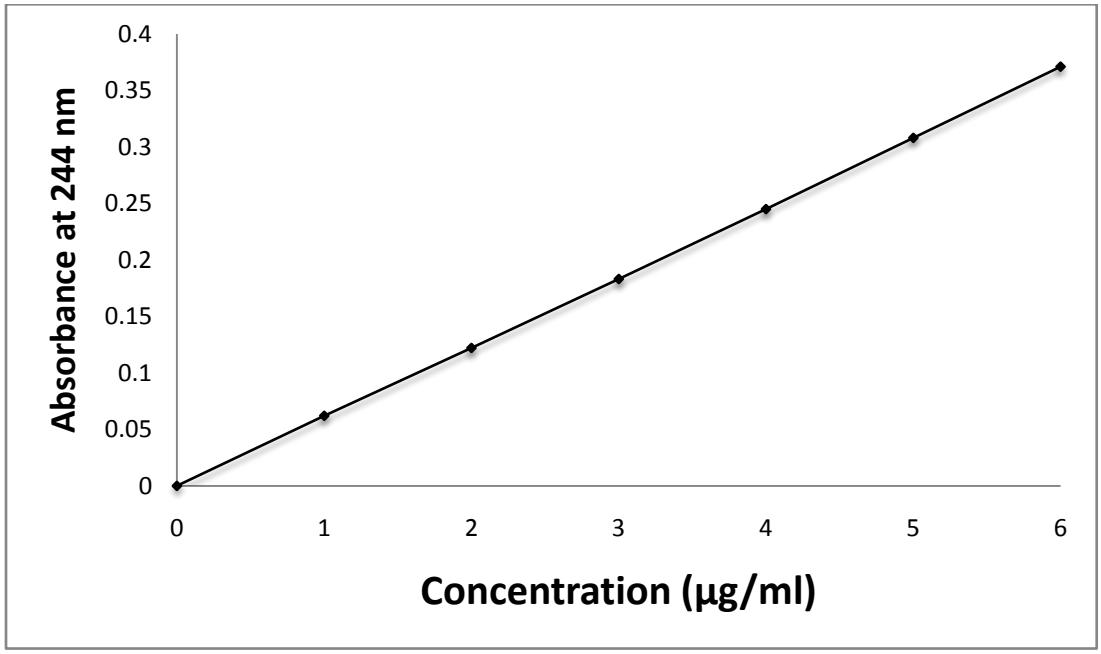


Figure 8.3: Calibration curve of Rosuvastatin calcium in methanol at 244 nm

Table 8.5:Data for calibration curve Parameters for 0.1 methanol

S.NO	PARAMETERS	VALUES
1	Correlation coefficient (r^2)	0.99996
2	Slope (m)	0.06171
3	Intercept(c)	-0.00071

Determination of λ max in 6.8 phosphate buffer:

The absorption maximum for Rosuvastatin in 6.8 phosphate buffer was found to be 240 nm and absorption maximum was shown in Figure 8.4

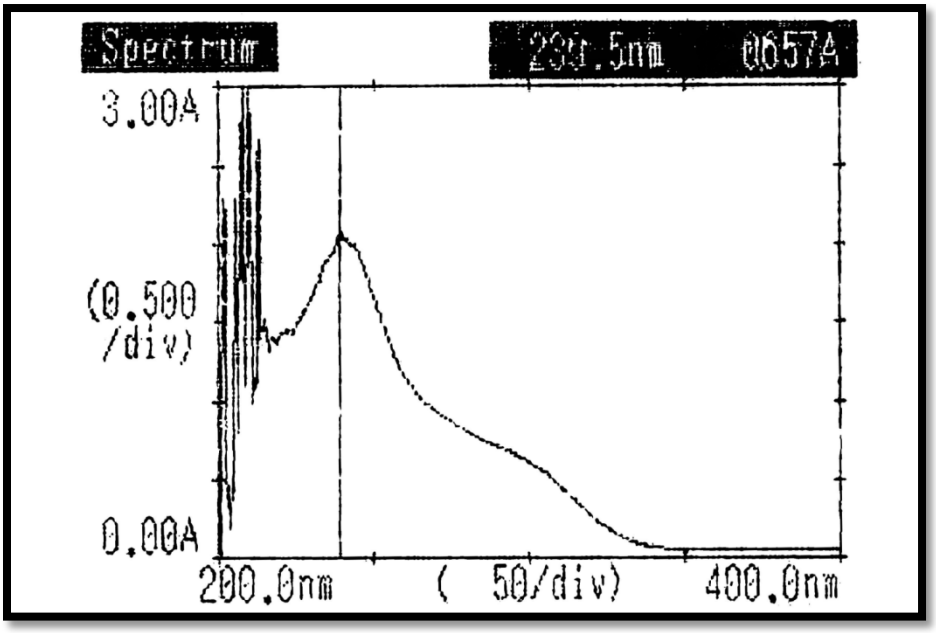


Figure 8.4: λ max observed for Rosuvastatin in 6.8 buffer

Preparation of standard graph of Rosuvastatin in 6.8 phosphate buffer:

Absorbance obtained for various concentrations of Rosuvastatin in6.8 phosphate buffer were given in Table8.6.and Figure 8.6 .The graph of absorbance vs concentration for Rosuvastatin was found to be linear in the concentration range of 10 µg /ml.The calibration curve parameters shown in Table 8.7.So the drug obeys Beer- Lambert's law in the range of 10 µg /ml.

Table 8.6: Calibration curve of Rosuvastatin calcium in 6.8 phosphate buffer at 240 nm

S.NO	Concentration (µg/ml)	Absorbance at 240nm
1	0	0
2	1	0.071
3	2	0.142
4	3	0.211
5	4	0.278
6	5	0.349
7	6	0.423

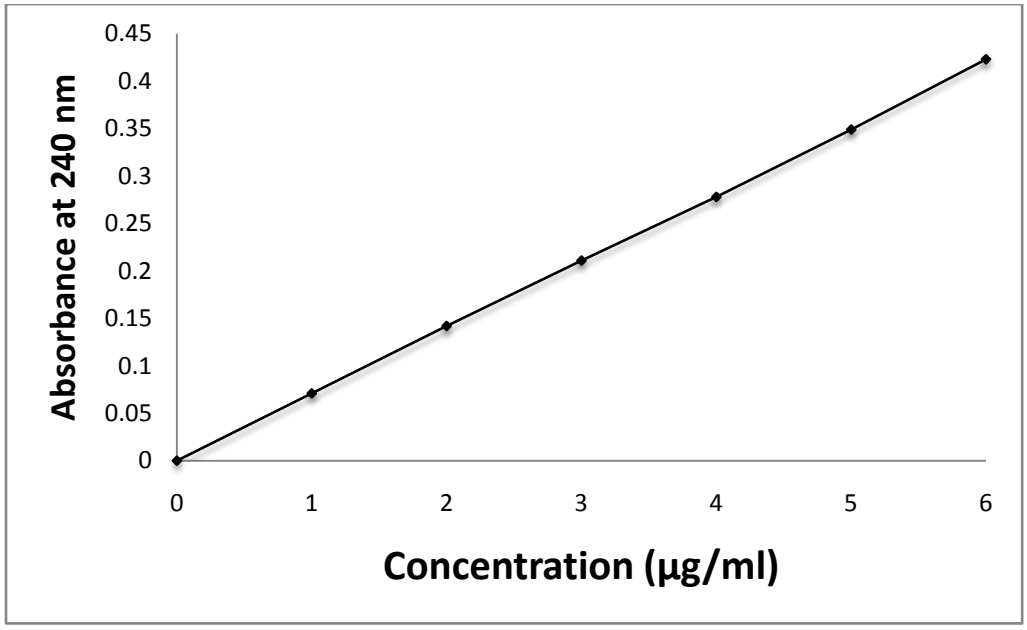


Figure 8.5: Calibration curveofRosuvastatin calcium in 6.8 buffer at 240 nm

Table 8.7: Data for calibration curve Parameters for in 6.8 phosphate buffer

S.NO	Parameters	Values
1	Correlation coefficient (r^2)	0.999936
2	Slope (m)	0.0070036
3	Intercept (c)	-0.000464

Percentage purity of drug

The percentage purity of drug was calculated by using calibration graph method (least square method).

Table 8.8: Percentage purity of drug

S. No	Percentage purity (%)	Average percentage purity (%)
1	99.10	99.07±1.24
2	99.53	
3	99.60	

All the values are expressed as mean± S.D., n=3.

The reported percentage purity for Rosuvastatin in IP 2007 is 98 to 99.53%.

Determination of compatibility for drug with polymer

By FTIR spectroscopy

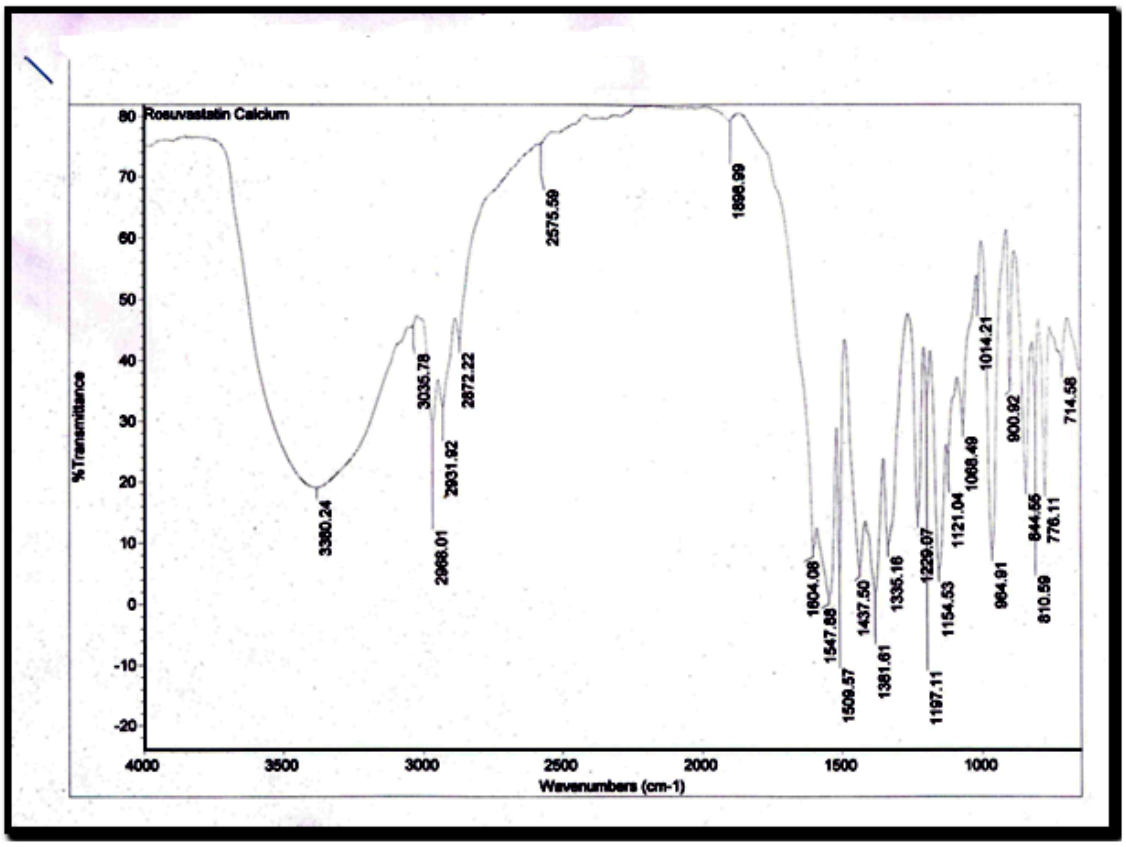


Figure 8.6:FTIR spectrum of Rosuvastatin

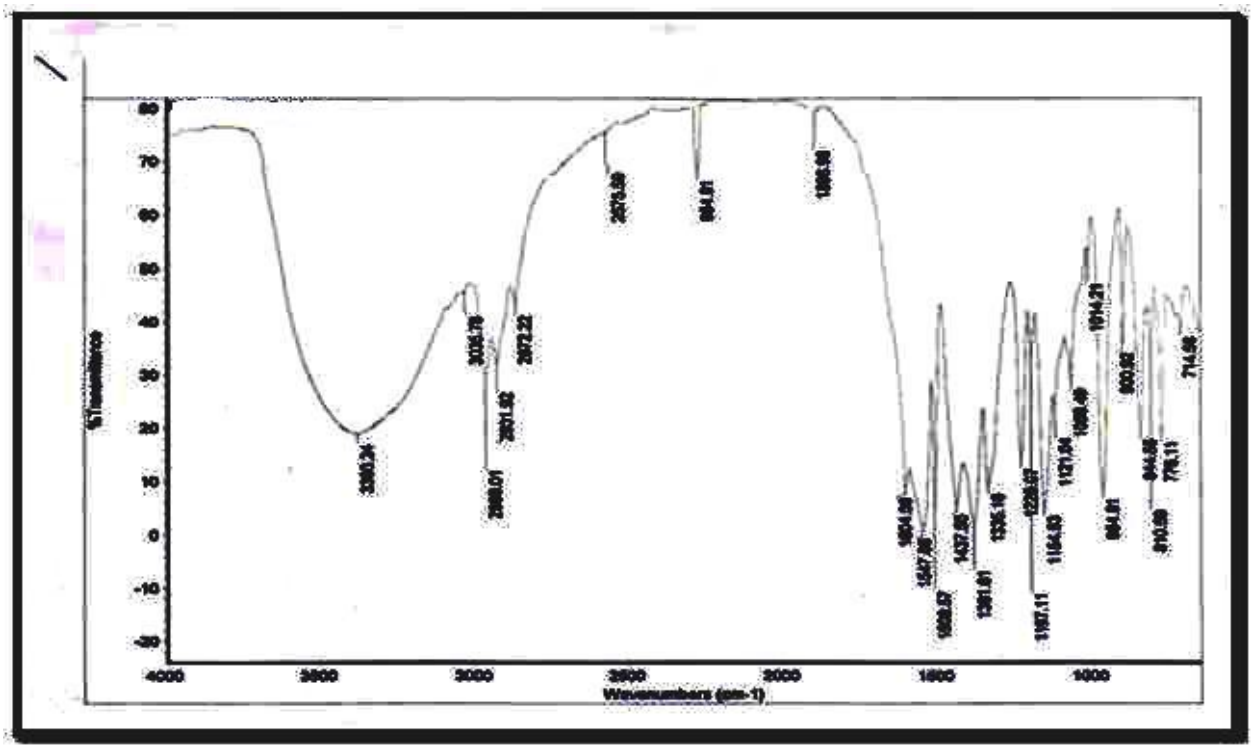


Figure 8.7: FTIR spectrum of drug and gelatin A.

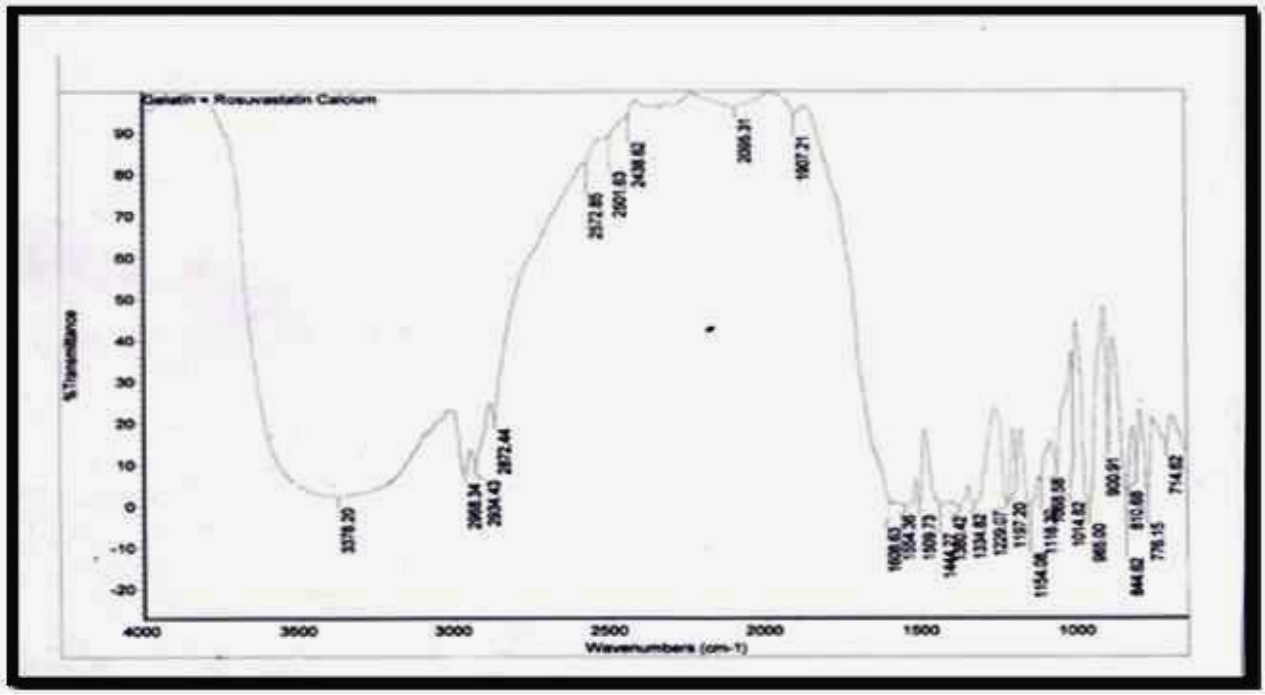


Figure 8.8:FTIR spectrum of drug and gelatin B.

Interpretation of FTIR spectrum:

Table 8.9: Interpretation of FTIR spectrum

S.NO	FORMULATION	WAVE NUMBER (CM^{-1})
1.	Pure Rosuvastatin	3380.24,3055.78,2968.01,2931.92,2872.22,2575.59,1898.99 1604.08,1547.88,1509.57,1437.50,1381.61,1335.16,1229.07,1197.11, 1154.53,1121.04,1068.49,1014.21,964.91,900.92,844.55,810.59,776.11, 714.58
2.	Gelatin	2959.12,2926.12,2855.03,2644.11,2400.33,2140.11,1981.23,1731.97,168 5.40,1630.59,1562.14,1510.10,1451.12,1406.01,1326.57,1245.14,1192.35 ,1156.55,1180.31,1022.55,970.48,918.44,871.87,773.26,721.22,668.20
3.	Rosuvastatin+ Gelatin	3378.20,2968.34,2934.43,2872.44,2572.85,2501.63,2438.62,2095.31,190 7.21,1608.63,1554.73,1444.27,1380.42,1334.82,1229.07,1197.20,1154.08 ,111.30,1068.58,1014.82,965.00,900.91,810.68,844.62,776.15,714.62

According to Table 8.9 and Figure 8.6 to 8.8.The major peaks observed in drug spectrum also observed in drug with polymerspectrum.ThereforeFTIR spectrums were compared; it could indicate that there was no incompatibility between drug and polymer.

By DSC thermal analysis

The compatibility and interactions between drugs and polymer were analysed byDSCthermogram and results were obtained represented and shown in Figure 8.9 to 8.11.

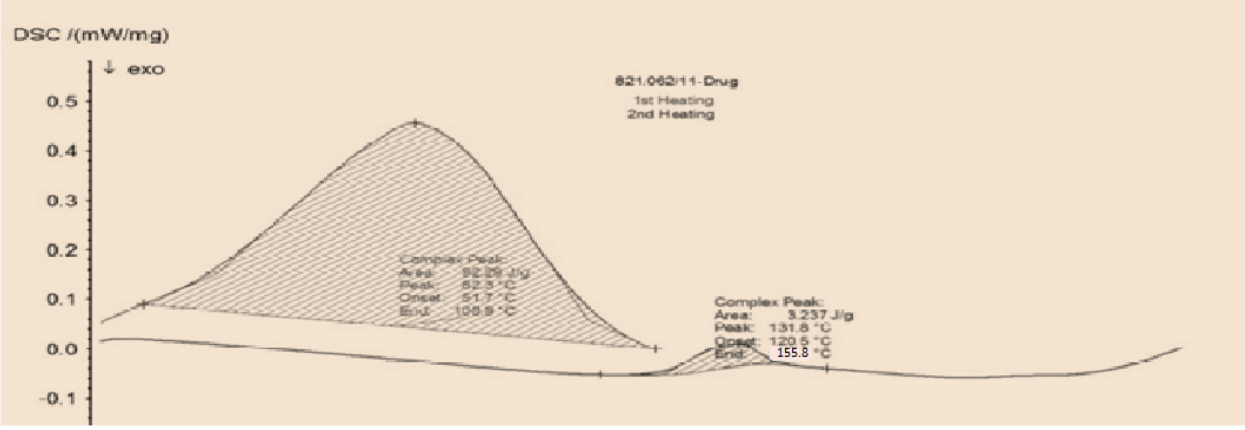


Figure 8.9: DSC thermo grams of Rosuvastatin

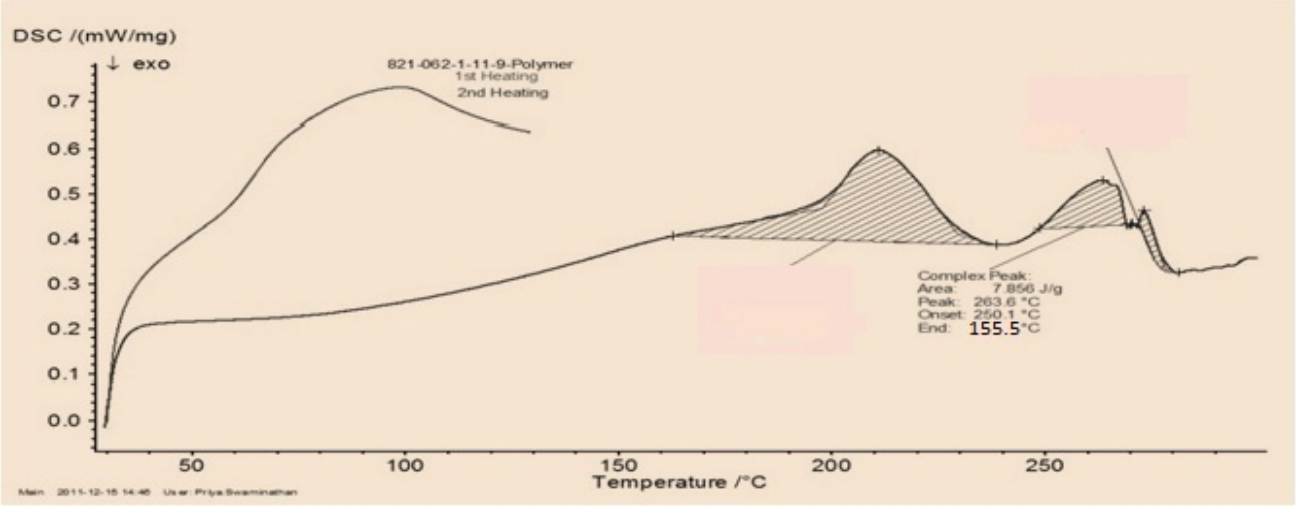


Figure 8.10:DSC thermo grams of Rosuvastatin+ gelatin A

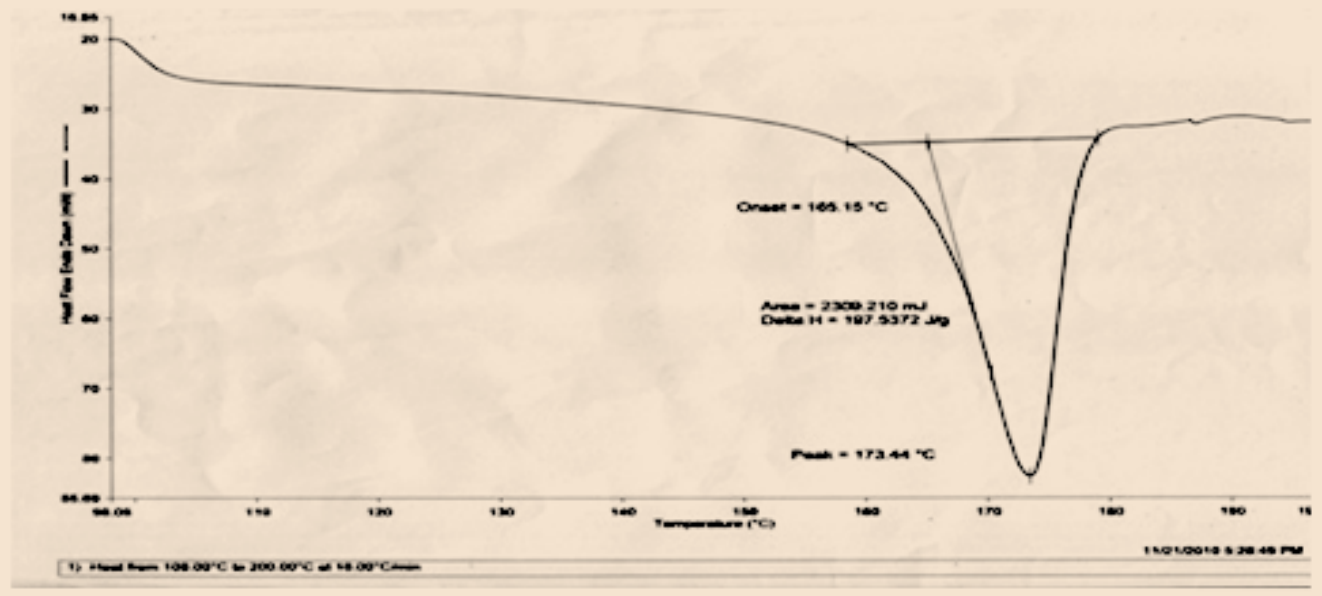


Figure 8.11:DSC thermo grams of Rosuvastatin+ gelatin B

The possible drug and polymer interaction can be studied by using DSC. The Rosuvastatin exhibits a sharp endometric peak 155.8 which is corresponding with its melting point at figure 8.10 and the Rosuvastatin + gelatin A and B exhibits the sharp endometric peak 155.5 at figure 8.9. Therefore when compared with pure drug thermo gram. No interaction was found between drug and polymers.

8.2. Characterization of nanoparticles

Drug loading efficiency (%le) and drug loading capacity (%LC):

The results were shown in Table 8.10 and Figure.8.12 to 8.13. It is revealed that the highest, %LE and %LC in the nanoparticle preparation were obtained.

Table 8.10: % LE and % LC of Rosuvastatin nanoparticles with gelatin A and gelatin B

S.NO	Parameter	Formulation (Gelatin A)				Formulation (Gelatin B)			
		F1	F2	F3	F4	F5	F6	F7	F8
1	Drug entrapment efficiency (LE %)	91.73	72.93	76.50	86.27	69.85	87.74	88.68	68.72
2	Drug loading capacity (Lc %)	66.21	55.62	49.41	39.54	65.22	54.14	61.33	47.54

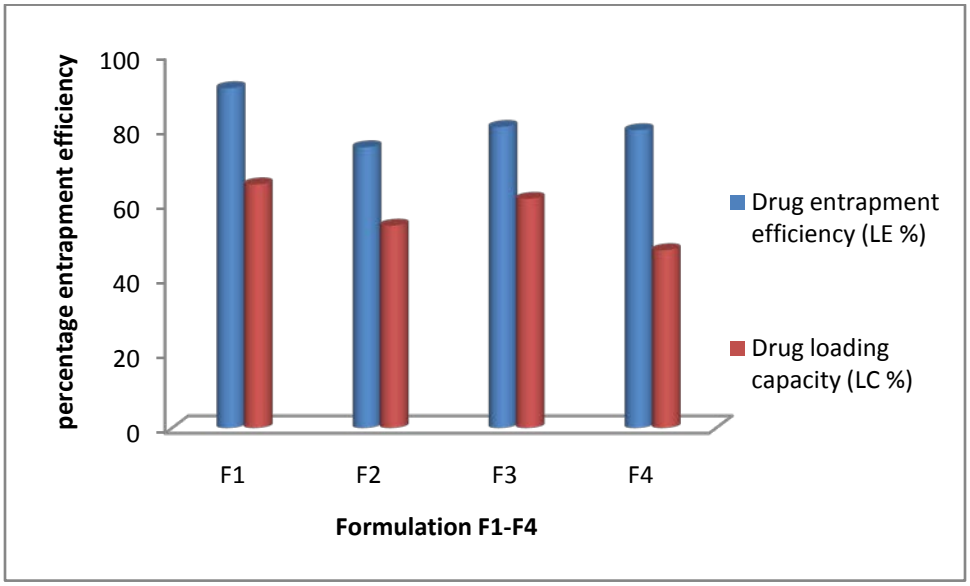


Figure 8.12:% LE and % LC of Rosuvastatin nanoparticles with gelatin A

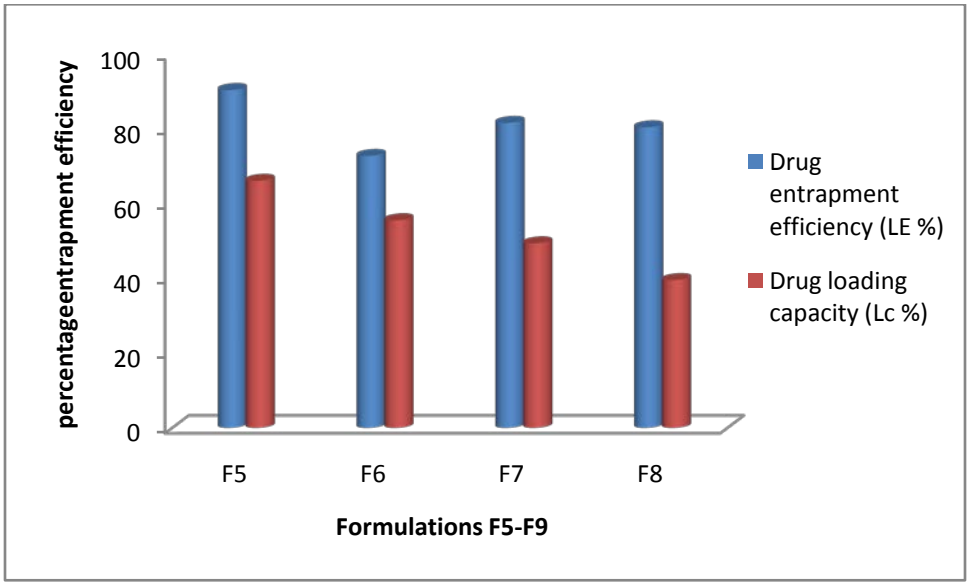


Figure 8.13:% LE and % LC of Rosuvastatin nanoparticles with gelatin B

Morphological characterization of F1 to F4 (gelatin A)

Rosuvastatin loaded polymeric nanoparticles were fabricated using*in-situ* two step desolvationmethodusing polymer Gelatin A.

The choice of an *in-situ* two step desolvation method of encapsulation was usually determined by the %yield, LE and %LC of the drug and sonication process which allowed reducing considerably the mean particle size and simultaneously to narrow the width of the size distribution, i.e. reduces the polydispersity index. The effectiveness of *in-situ* two step desolvation method and polymer on nanoparticle preparation was confirmed by their size, *in-vitro* release characteristics.

Morphological characterization of F5to F8 (gelatin B)

Rosuvastatin loaded polymeric nanoparticles were fabricated using*in-situ* two step desolvation method using polymer Gelatin B.

The choice of a of two step desolvation method encapsulation was usually determined by the solubility characteristics of the drug and high pressure homogenization process which allowed reducing considerably the mean particle size and simultaneously to narrow the width of the size distribution, i.e. reduced the polydispersity index. The effectiveness of two step desolvationmethod and polymer, on nanoparticle preparation was confirmed by their size,*in-vitro* release characteristics.

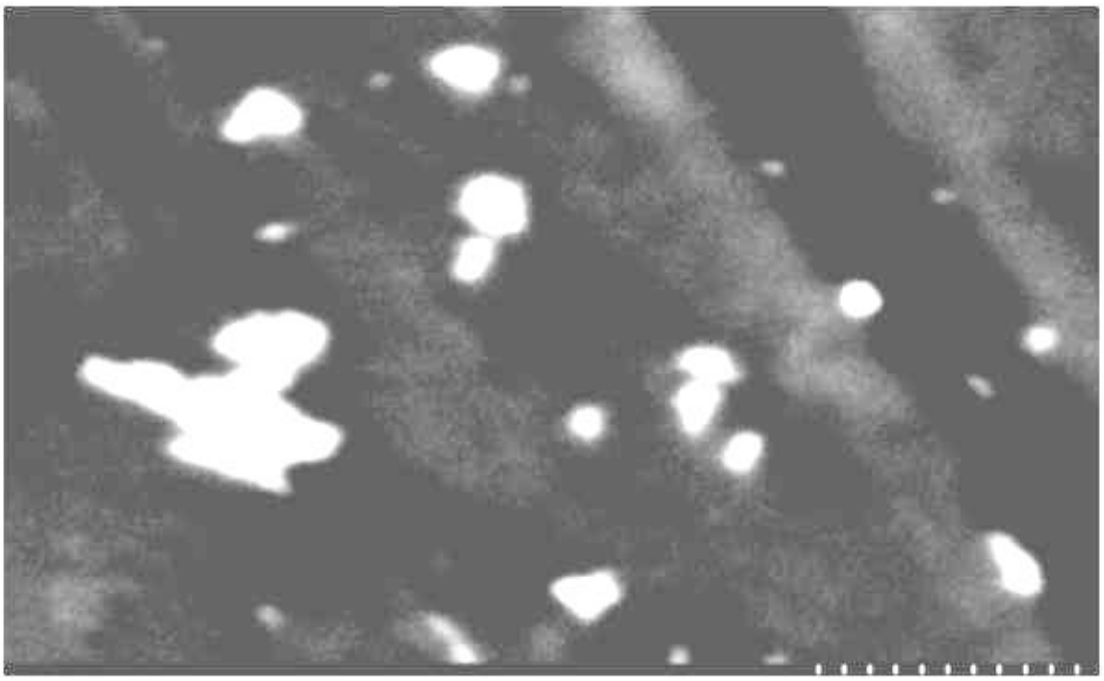
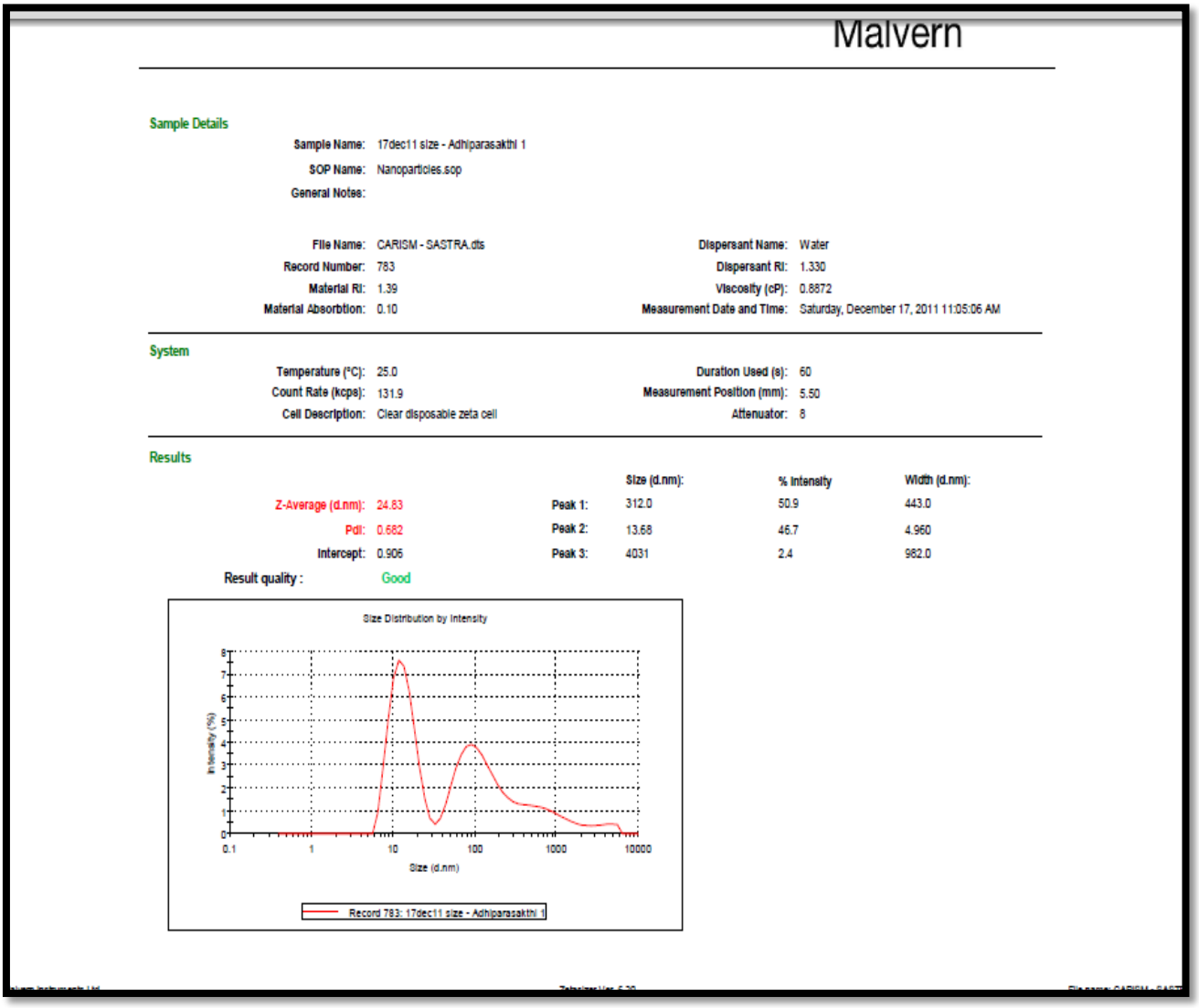


Figure 8.14: Scanning electron microscopy image of F1



Malvern

Sample Details

Sample Name:	17dec11 size - Adhiparasakthi 1		
SOP Name:	Nanoparticles.sop		
General Notes:			
File Name:	CARISM - SASTRA.dts	Dispersant Name:	Water
Record Number:	783	Dispersant RI:	1.330
Material RI:	1.39	Viscosity (cP):	0.8872
Material Absorbtion:	0.10	Measurement Date and Time:	Saturday, December 17, 2011 11:05:06 AM

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	131.9	Measurement Position (mm):	5.50
Cell Description:	Clear disposable zeta cell	Attenuator:	8

Results

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	24.83	Peak 1:	312.0	50.9	443.0
PdI:	0.682	Peak 2:	13.68	46.7	4.960
Intercept:	0.906	Peak 3:	4031	2.4	982.0

Result quality : Good

Size Distribution by Intensity

Intensity (%)

Size (d.nm)

Record 783: 17dec11 size - Adhiparasakthi 1

Figure 8.15: Particle size distribution F1

In-vitro Drug Release profile

In-vitro drugreleased profiles of Rosuvastatin nanoparticles were performed in each formulation dipped in the phosphate buffer (6.8 pH) upto 16 hours. It was represented in Table 8.11 and showed in Figure 8.16 to 8.24.

Table 8.11 Percentage in vitro drug released of Formulations F1-F8

S. No	Time in hours	pH medium	Gelatin A Percentage drug released(%)				Gelatin B Percentage drug released (%)			
			F1 %	F2 %	F3 %	F4 %	F5 %	F6 %	F7 %	F8 %
1	2	6.8 phosphate buffer	11.49± 0.11	12.83± 0.32	9.26±0. 37	10.60± 0.52	9.71±0. 65	7.48±1. 87	8.37±0. 25	13.50± 0.89
2	4		21.76± 0.22	20.86± 0.23	16.8±0. 25	16.62± 0.35	19.30± 0.44	15.95± 0,21	17.29± 0.87	22.87± 1.25
3	6		33.13± 0.52	32.46± 0.36	30.9±1. 25	30.01± 0.54	31.35± 0.87	28.45± 0.78	29.34± 0.54	31.39± 1.22
4	8		48.30± 0.17	43.84± 0.21	44.07± 0.36	44.96± 0.58	47.63± 1.58	45.85± 0.25	46.03± 0.77	45.40± 0.57
5	10		63.47± 0.33	61.02± 0.87	62.81± 0.14	61.98± 1.25	64.14± 0.47	62.14± 0.74	63.44± 0.52	63.92± 0.11
6	12		79.76± 0.54	78.20± 1.22	75.97± 0.87	74.41± 1.74	77.31± 1.87	76.41± 1.25	75.08± 1.58	75.97± 1.87
7	14		87.87± 0.58	85.56± 1.25	79.09± 0.65	81.99± 0.98	85.78± 1.22	84.89± 0.78	83.11± 1.32	82.66± 0.85
8	16		93.38± 1.20	92.25± 0.44	88.24± 1.47	84.89± 0.14	92.49± 0.57	91.59± 0.87	87.35± 0.58	86.68± 0.74

All the values were expressed as mean ± SD., n=6

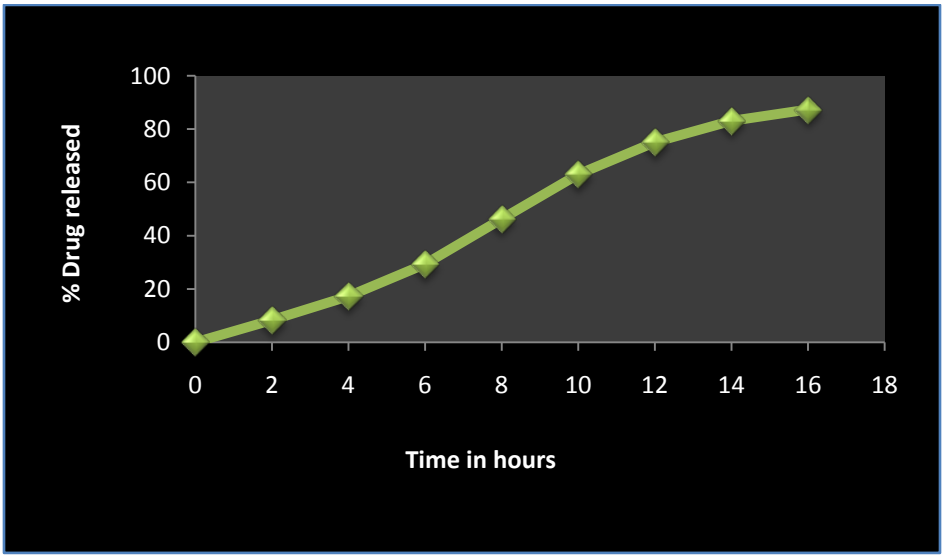


Figure 8.16: Graphical representation of *invitro* percentage drug released for formulation F1

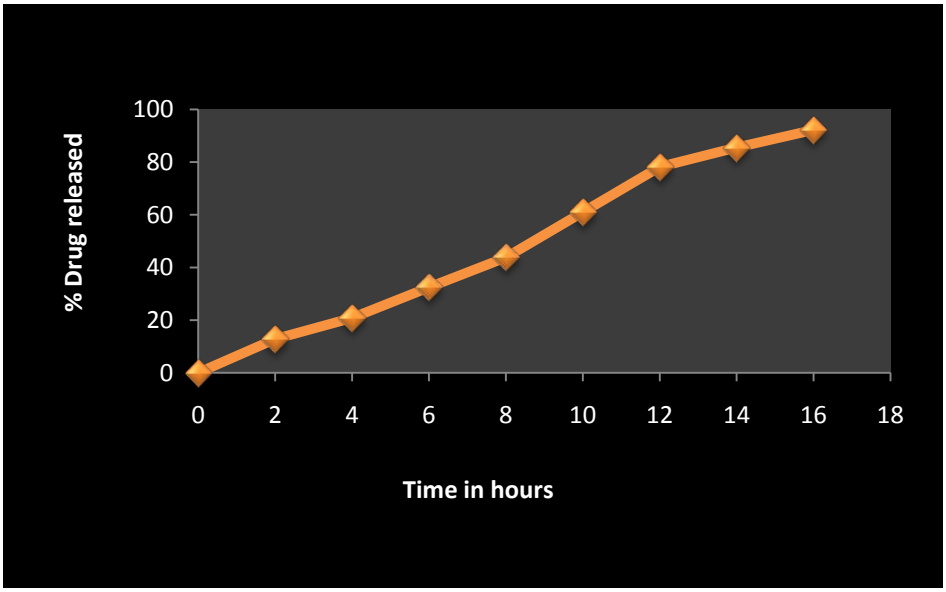


Figure 8.17: Graphical representation of *invitro* percentage drug released for formulation F2

/

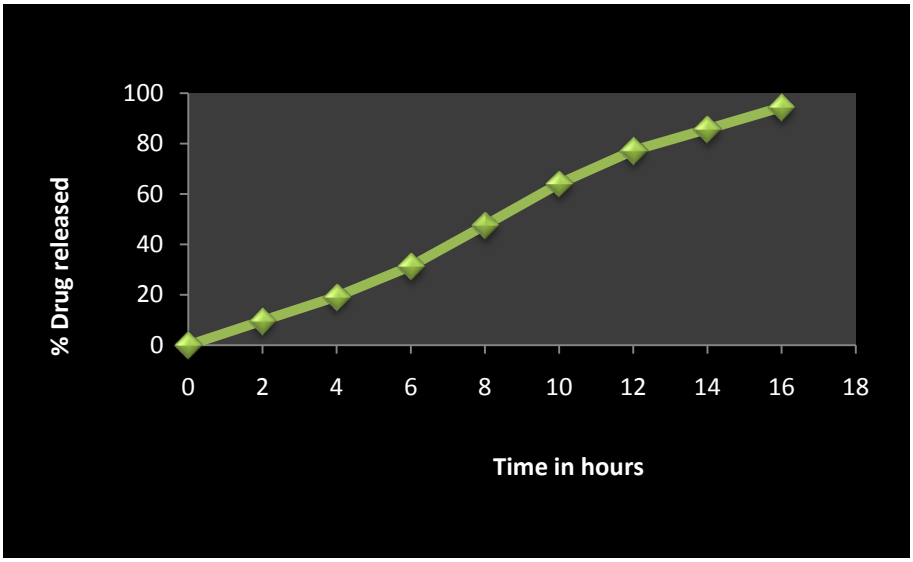


Figure8.18: Graphical representation of *invitro* percentage drug released for formulation F3

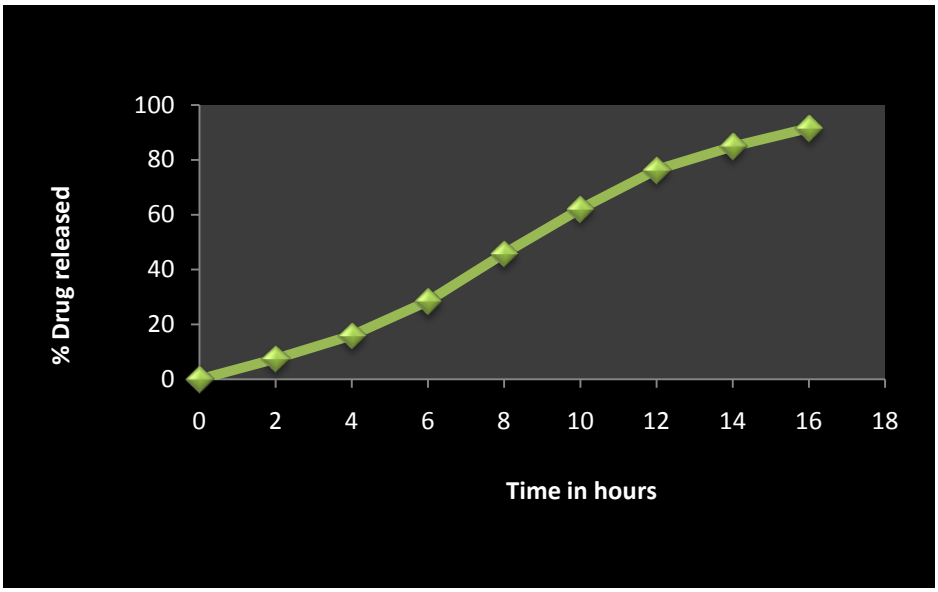


Figure8.19: Graphical representation of *invitro* percentage drug released forformulation F4

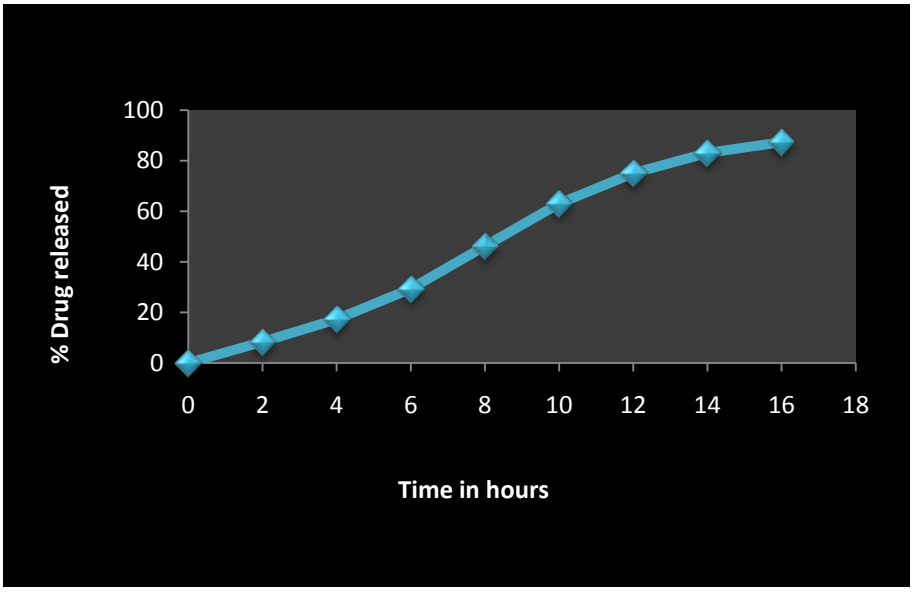


Figure 8.20: Graphical representation of *invitro* percentage drug released for formulation F5

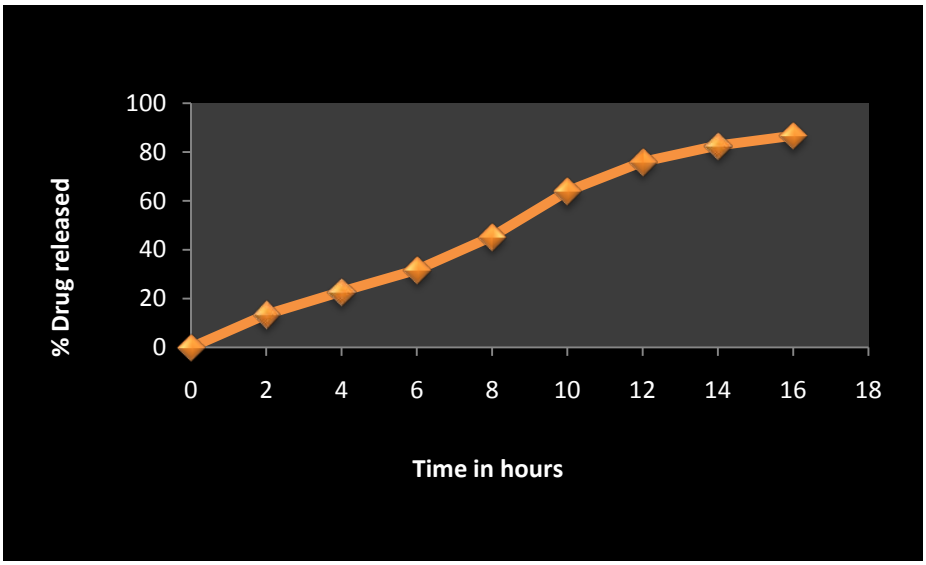


Figure 8.21: Graphical representation of *invitro* percentage drug released for formulation F6

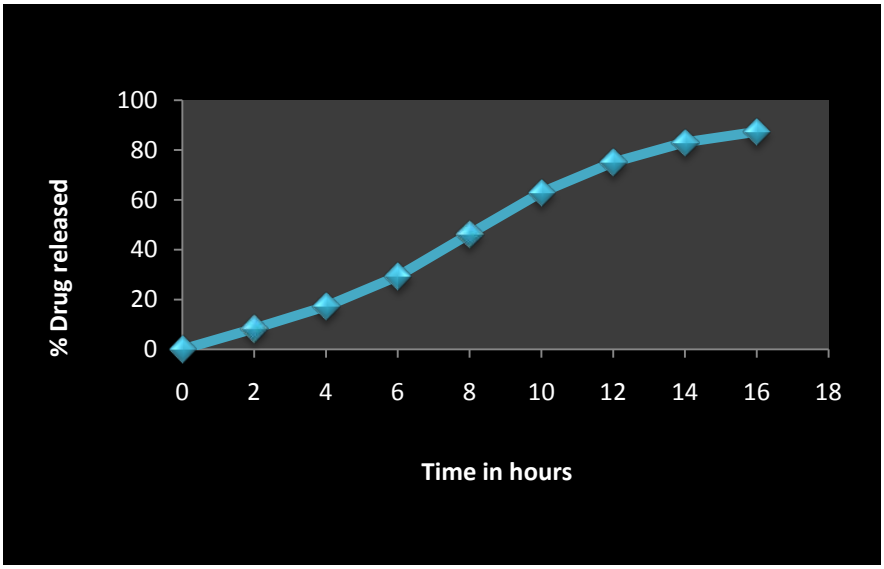


Figure 8.22: Graphical representation of *invitro* percentage drug released for formulation F7

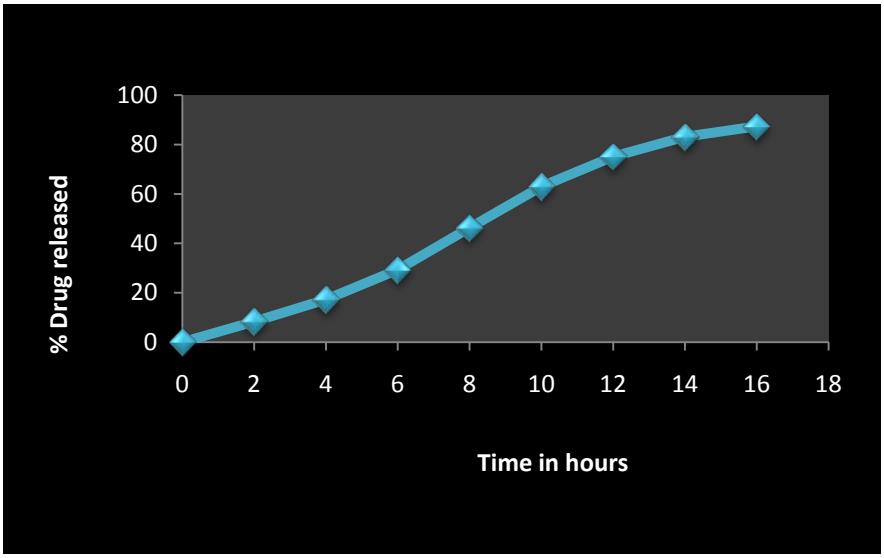


Figure 8.23: Graphical representation of *invitro* percentage drug released for formulation F8

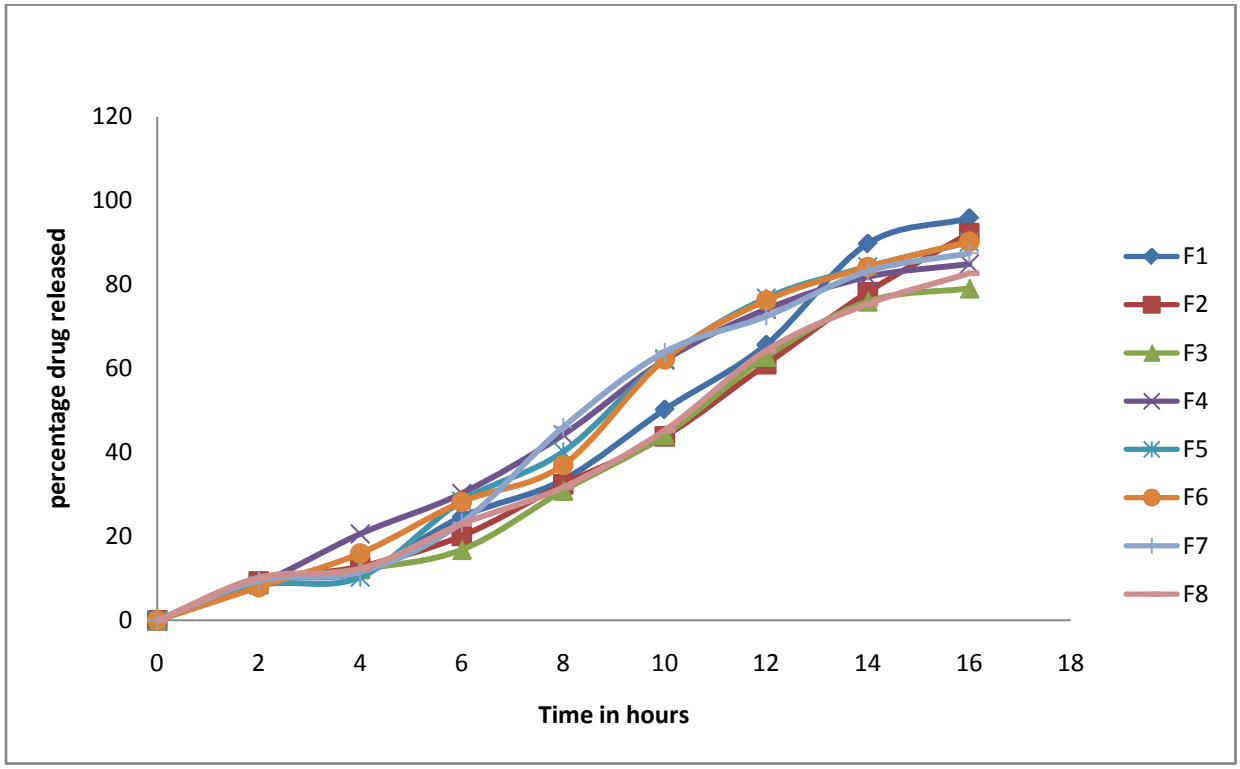


Figure 8.24: Graphical representation of comprehensive *invitro* percentage drug released for formulations F1-F8

Release Kinetics of *In-vitro* Drug Release

The kinetics of *in-vitro* drug released was determined by applying the drug release data to various kinetic models such as zero order first order, Higuchi and Korsmeyer- Peppas. The results obtained were represented Table 8.12 and Figure 8.25 to 8.32.

Table 8.12: Release kinetics of *In-vitro* drugrelease gelatin A and gelatin B

Formulations	Zero order R^2	First order R^2	Higuch R^2	Peppas R^2	Best Fit Model
F1	0.991	0.845	0.978	0.837	Zero
F2	0.986	0.873	0.974	0.734	Zero
F3	0.983	0.914	0.975	0.924	Zero
F4	0.981	0.923	0.968	0.864	Zero
F5	0.992	0.838	0.976	0.877	Zero
F6	0.989	0.870	0.964	0.962	Zero
F7	0.983	0.907	0.973	0.821	Zero
F8	0.984	0.929	0.968	0.912	Zero

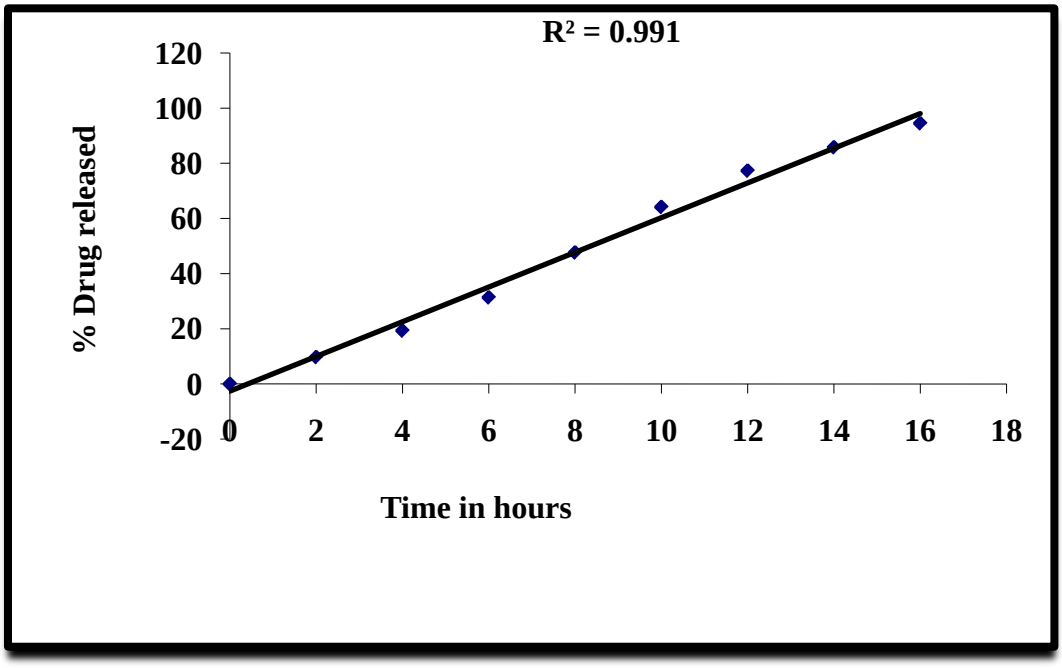


Figure 8.25: Best fit model (zero order) of formulation F1

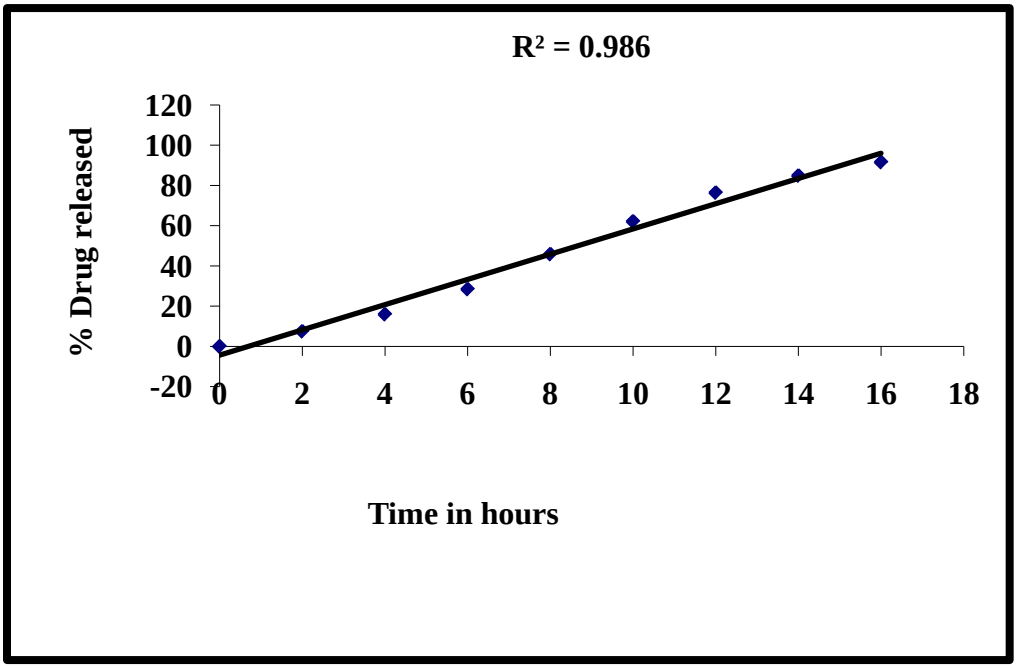


Figure 8.26: Best fit model (zero order) of formulation F2

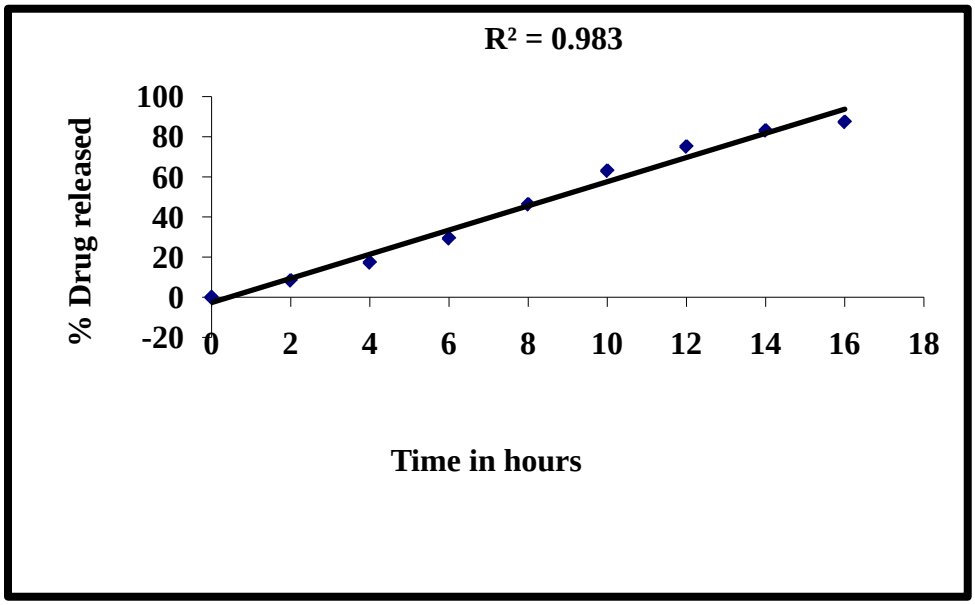


Figure 8.27 Best fit model (zero order) of formulation F3

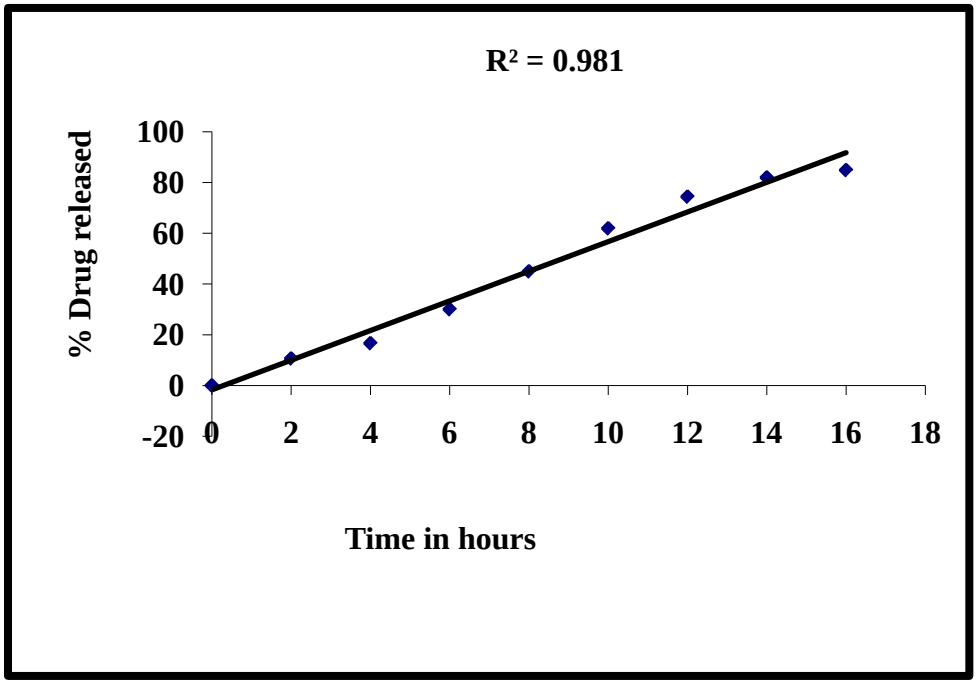


Figure 8.28: Best fit model (zero order) of formulation F4

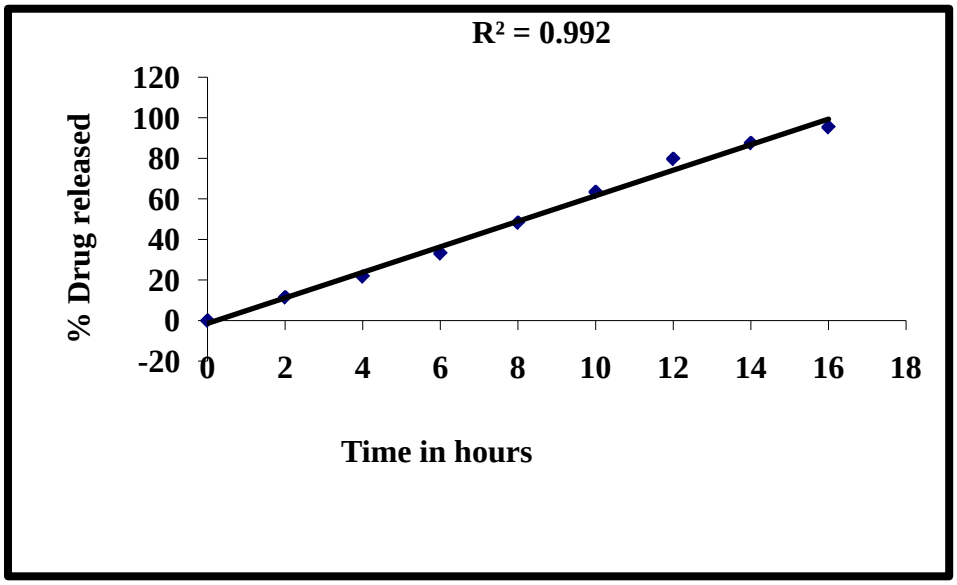


Figure 8.29: Best fit model (zero order) of formulationF5

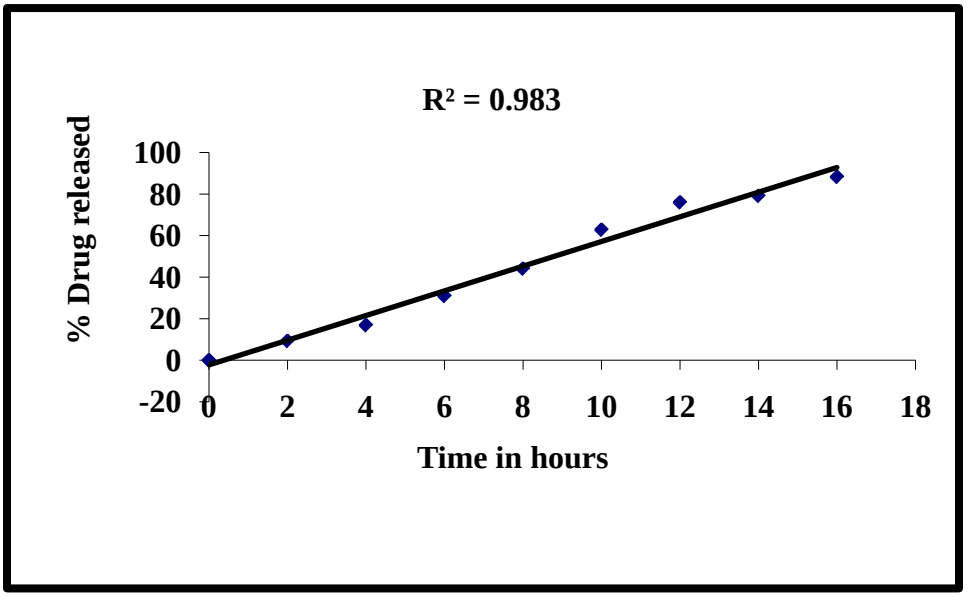


Figure 8.30: Best fit model (zero order) of formulation F6

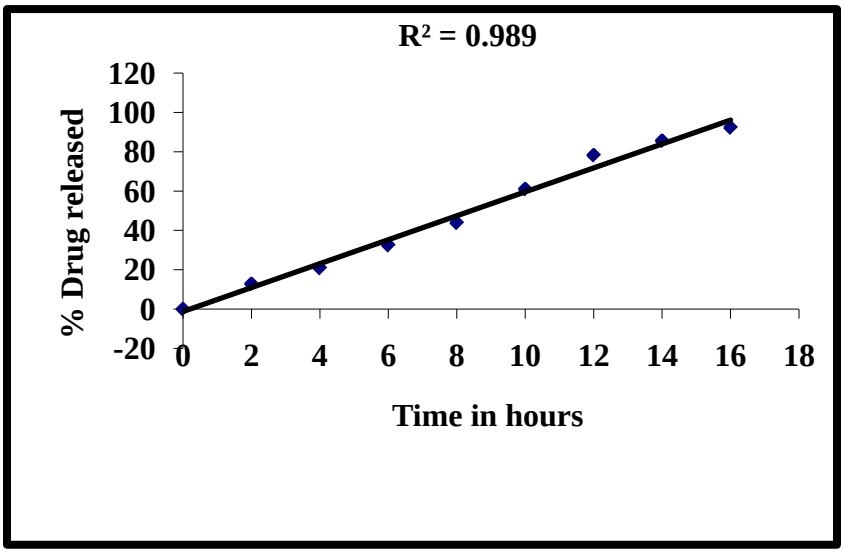


Figure 8.31: Best fit model (zero order) of formulation F7

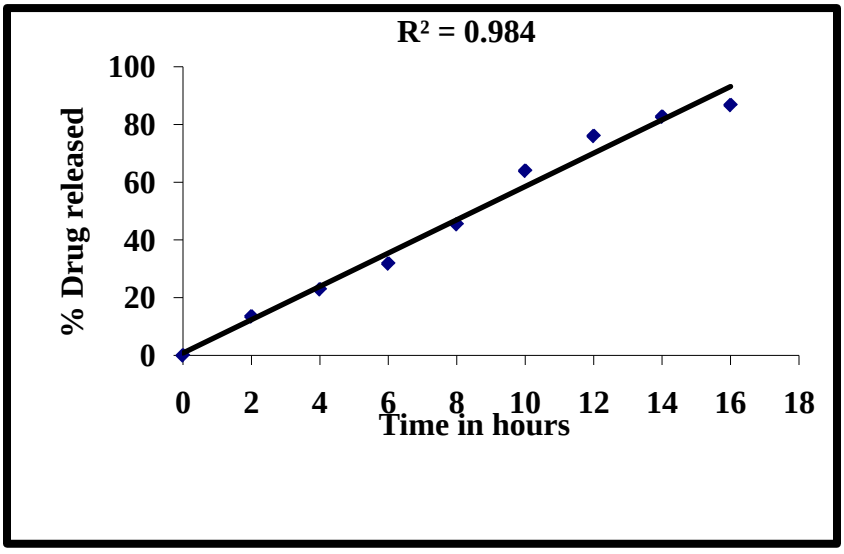


Figure 8.32: Best fit model (zero order) of formulation F8

From the data and graphical representations, the Rosuvastatin nanoparticles formulations were showed well fitted zero order kinetics and formulation F1 was showed best among the formulations were prepared based on morphology, %drug loading efficiency, *in-vitro* drug released profiles and also well fitted the zero order kinetics.

8.3 Stability studies

FormulationF1was kept in room temperature**,** accelerated condition and refrigerator temperature. After exposed to specified periods of time the samples were analyzed for %LE. Results were represented in Table 8.13

Table 8.13: Stability studies of optimized formulation (F1).

S.No	Temperature(0^0c)	Drug loading efficiency(%LE)		
		1st month	2nd month	3rd month
1	25^0C±2^0C at RH 60%±5%	98.45	98.98	97.93
2	40^0C±2^0C at RH 75%±5%	98.55	96.80	96.92
3	5^0C±3^0C	98.29	98.14	97.35

No major difference was found between %LE before and after stability studies.The formulation F1 was showed satisfactory physical stability at 25^0C ±2^0C at RH 60%±5%, 40^0C ±2^0C at RH 75%±5% and 5^0C±3^0C.

8.4. Zeta potential

The zeta potential report of the formulation F1 showed in Figure 8.33. The obtained value was the positive value.

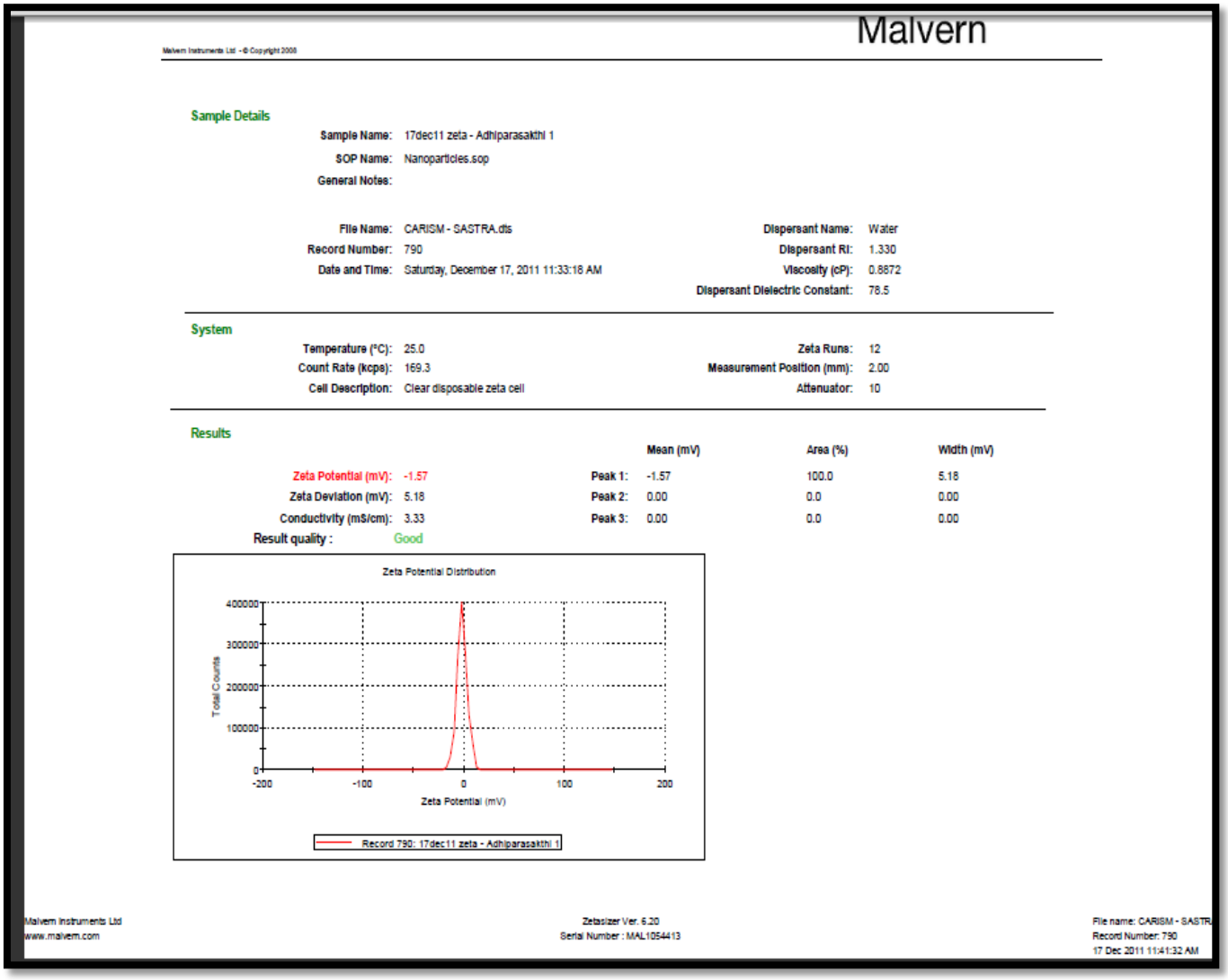


Figure 8.33: Zeta potential of F1 formulation

SUMMARY

AND

CONCLUSION

9. SUMMARY AND CONCLUSION

The Rosuvastatin was widely used in the antilipedimic drug.This research work mainly focused on therapeutic effect of the drug to increased Bioavailability. The reported Bioavailability of Rosuvastatin was only 20%. While forming the novel drug delivery system in the form of nanoparticle to increased the bioavalability.

Eight formulations were prepared by using drug with gelatin A and gelatin B in different ratio of 1:1,1:2,1:3 and1:4 each polymer respectively(F1 to F8). Among the eight formulations F1 formulation was best because of these formulation shows the optimized result of colloidal drug delivery systems of nanoparticles have emerged as an efficient means enhancing the bioavailability with lowest possible dose.

The present work was proposed to prepared nanoparticles loaded with Rosuvastatin to achieved better bioavailability with low dose of the drug at the site, decreased the risk of adverse side effects. Rosuvastatin nanoparticles were prepared by *in-situ* two step desolvation method.

The loading efficiency(F1) and Entrapment efficiency(F1) was 66.21 and 91.73 respectively.

The shape of nanoparticle was found to be spherical by SEM analysis. Formulation with high polymer content was observed to be fairly spherical. Compatability of drug and polymer mixture was done by performing FTIR and DSC study. It was concluded that there was no interaction between drug and polymer.

The *in-vitro* released of Rosuvastatin was evaluated in phosphate buffer saline (pH6.8) up to 16hrs. The formulation F1 was released the drug 93.38% up to16 hours. and chosen the best among the formulations were prepared.

Zeta potential was determined for the formulation F1 and it was found to be +ve value of 1.57mv.

The *in-vitro* drug released data was applied to various kinetic models like zero order kinetics, Higuchi plot, first order kinetics, and Peppas plot by predict the drug release kinetics mechanism. The formulation F1 was best fitted the zero order kinetics.

In short term stability studies the formulation F1 was showed that there was no remarkable changes in the % drug loading efficiency.

Based on the % drug loading efficiency, zeta potential, *in-vitro* drug release profile, *in-vitro* drug release kinetics and stability studies the formulation F1 was found to be best one among the formulations (F1 to F8) were prepared.

These nanoparticles can be promising agents for rational drug delivery in lipidemic condition.

FUTURE PROSPECTS

10.FUTURE PROSPECTS

1. Nano-drug delivery systems that deliver large but highly localized quantities of drugs to specific areas to be released in controlled ways.
2. Controllable release profiles, especially for sensitive drugs.
3. Materials for nanoparticles that are biocompatible and biodegradable.
4. Architectures / structures, such as biomimetic polymers, nanotubes.
5. Technologies for self-assembly.
6. Advanced polymeric carriers for the delivery of therapeutic peptide/proteins (biopharmaceutics).

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