Formulation and Evaluation of Olmesartan Loaded Solid Lipid Nanoparticles for Enhancing Oral Bioavailability

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SUBMITTED BY-

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I hereby declare that the matter embodied in the dissertation entitled "**Formulation and Evaluation of Olmesartan Loaded Solid Lipid Nanoparticles for Enhancing Oral Bioavailability**" is a bonafide and genuine research work carried out by me under the supervision of **Dr. Sheikh Sofiur Rahman**, Assistant Professor, Dept. of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati. The work embodied in this thesis is original and has not been submitted the basis for the award of any degree, diploma or fellowship in any other university or institution.

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ABSTRACT

Drugs with low aqueous solubility not only give low oral bioavailability but provide high inter-and intrasubject variability. Olmesartan medoxomil has very poor aqueous solubility and belongs to Class II drugs under Biopharmaceutical Classification Systems. The purpose of the present study was to investigate the bioavailability enhancement of olmesartan medoxomil by solid lipid nanoparticles. Optimized blank solid lipid nanoparticles were prepared by hot homogenization and ultra-sonication method using Stearic acid, Soya lecithin, Chloroform, Methanol, Poloxamer 188. Optimization was done by particle size and polydispersity index. The λ_{max} of the Olmesartan medoxomil was found to be 257nm in methanol. The physicochemical compatibility study was carried out by infrared spectroscopy. After the interpretation drug and drug-polymer mixture was found to be compatible. Previous report had shown that particle sizes less than 300 nm are advisable for the intestinal transport. It has been reported that a polydispersity index value less than 0.3 is often accepted as optimum value. So from the obtained result of this present study, it is concluded that the blank formulation with rpm 12000 for 4 min was better formulation with particle size 203.5 nm and PDI 0.228. Hence this rpm with that specific time will be suitable parameters to suggest in carrying out the formulations of Olmesartan loaded solid lipid nanoparticles.

Keywords: Olmesartan medoxomil; Solid lipid nanoparticles; Homogenization; Ultra-Sonication; Polydispersity index; Infrared spectroscopy

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ABBREVIATIONS	DESCRIPTIONS
BCS	Biopharmaceutical Classification System
GIT	Gastrointestinal Tract
p^H	Hydrogen ion concentration
ACE	Angiotensin Converting Enzyme
Hg	Inch of mercury
NPs	Nanoparticles
CNTs	Carbon nano-tubes
PNP	Polymer nanoparticle
SLNs	Solid Lipid Nanoparticles
HPH	High pressure homogenization
PDI	Polydispersity index
FDA	Food and Drug Administration
TEM	Transmission Electron Microscopy
DNA	Deoxyribonucleic acid
NLC	Nanostructured Lipid Carriers
PVA	Poly vinyl alcohol
OLM	Olmesartan medoxomil
PVP	Polyvinylpyrrolidone
NS	Nanosuspensions
DSC	Differential Scanning Calorimetry

FT-IR	Fourier transform infrared spectroscopy
API	Active pharmaceutical ingredient
EE	Entrapment Efficiency
HPLC	High performance liquid chromatography
Hcl	Hydrochloric acid
IAEC	Institutional Animal Ethical Committee
C_{max}	Maximum plasma concentration of the drug
UV	Ultraviolet
λ_{max}	Wavelength of maximum absorbance

- **LIST OF UNITS**

UNITS	DESCRIPTION
°C	Degree Celsius
hrs	Hours
min	Minute
%	Percentage
mg	Miligram
g	Gram
ml	Mililiter
µl	Microliter
mm	Milimeter
nm	Nanometer
mg/ml	Miligram/ Mililiter
µg/ml	Microgram/ Mililiter
cm^{-1}	Inverse Centimeter
w/w	Weight/ Weight
v/v	Volume/ Volume

CHAPTER - 1

INTRODUCTION

1. INTRODUCTION

Oral delivery of a drug is the most convenient, common, and widely used route of administration for compliance like effortless administration, no assistance, patient conformity as compared to the other routes; for example intravenous, pulmonary, and intramuscular drug delivery. Because of the low bioavailability and low absorption upon oral administration several compounds failed and did not succeed in the research and development (1). The drugs which have poor oral bioavailability are not capable to reach the minimum effective concentration to exhibit the therapeutic action (2). The reasons for the poor bioavailability of the drugs depend on some parameters like dissolution rate, permeability, aqueous solubility, susceptibility to efflux mechanisms, first-pass metabolism. In recent years, almost 70% of new drug candidates are showing poor bioavailability (3).

Although oral drug delivery is effective for drugs with high aqueous solubility and epithelial permeability, efficient oral administration of the poorly water-soluble drug may be a challenge. Presently, most of the new chemical entities are lipophilic and consequently have poor aqueous solubility (4). On the idea of the biopharmaceutical classification system (BCS), a variety of latest therapeutic entities are characterized under BCS class II (low solubility and high permeability) or BCS class IV (low solubility and low permeability). Besides, the oral bioavailability of certain drugs is also suffering from their poor gastrointestinal permeability. To achieve effective therapeutic action, these drugs need to be given at a high dose for example antiviral drugs. Moreover, chemical and enzymatic barriers presented by the gastrointestinal tract (GIT) also affect the oral administration of medicine. The change in GIT p^H and the presence of sort of enzymes significantly affect the oral bioavailability of medicine like

antihypertensive, antibiotics, antihyperlipidemic agents, and so forth. Furthermore, drugs with high first-pass metabolism such as repaglinide, β-blockers, calcium channel blockers, and ACE inhibitors even have low oral bioavailability. These drugs also present a challenge in formulation development for oral administration (5).

1.1 HYPERTENSION:

Hypertension is a chronic illness associated with high morbidity and mortality. Once hypertension is diagnosed, starting antihypertensive therapy on a long-term basis along with regular follow up is important. High blood pressure is defined as a systolic blood pressure (BP) ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg. The diastolic pressure represents the pressure during ventricular relaxation in diastole whereas the systolic pressure represents the peak pressure due to ventricular contraction during systole.

- **Signs and Symptoms of Hypertension:** The primary hypertension patient may be generally asymptomatic or may impose serious cardiovascular disease risk factors, perhaps America Heart Association, mentions cardiovascular risk factors associate with age, gender, heredity, smoking, high lipid profile, obesity and overweight, Diabetes mellitus. Adult patients with an average of two or more previous elevated blood pressure readings (6).
- **Causes of Hypertension:** Despite the fact that the definite reasons for hypertension are generally obscure, there are a few variables that have been very connected with the condition. These include:
  - ✓ Smoking
  - ✓ Obesity or being overweight
  - ✓ Being stout/overweight as a kid
  - ✓ Diabetes

- ✓ Sedentary way of life
- ✓ Lack of physical action
- ✓ High levels of salt admission (sodium affectability)
- ✓ Insufficient calcium, potassium, and magnesium utilization
- ✓ Vitamin D lack
- ✓ High levels of liquor utilization
- ✓ Stress
- ✓ Aging
- ✓ Medicines, for example, conception prevention pills
- ✓ Genetics and a family history of hypertension (a few qualities in the kidneys may add to hypertension)
- ✓ Chronic kidney sickness
- ✓ Adrenal and thyroid issues or tumors (7).

- **Classification of Antihypertensive Drugs:**

Presently available anti-hypertensive drugs can be classified into the following categories-

a) ACE inhibitors,

b) Angiotensin antagonist,

c) Calcium channel blocker,

d) Diuretics,

e) Central sympatholytics,

f) α-Adrenergic blocker,

g) Vasodilator,

h) β- Adrenergic blocker.

The majority of these drugs bear a few significant drawbacks like low permeability, low bioavailability, comparatively short half-life, and undesirable side effects. To overcome such types of challenges related to antihypertensive drug therapy, novel drug delivery systems present an opportunity for formulation scientists which can provide the following characteristics:

a) Enhanced bioavailability,
b) Low dosing frequency,
c) Reduced side effects, and
d) Increased selectivity (8).

1.2 NANOPARTICLES:

Since last century, Nanotechnology is a well-known field of research. Nobel laureate Richard P. Feynman presented "Nanotechnology" during his well famous 1959 lecture "There's Plenty of Room at the Bottom" and there have been made various revolutionary developments in the field of nanotechnology. Nanotechnology produced various types of materials at nano-scale level. Nanoparticles (NPs) are extensive class of materials that include particulate substances having size ranges from 1 to 100 nm(9). Nanoparticles are not simple molecules itself and thus composed of three layers:

a) The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants and polymers.
b) The shell layer, which is chemically different material from the core in all aspects, and
c) The core, which is essentially the central portion of the NP and usually refers the NP itself.(10)

- **Classification of nanoparticles:**

Depending on their size, morphology, and chemical properties, NPs are generally divided into various categories. Based on chemical and physical features, some of the well known classes of NPs are given as below:

i. **Carbon-based Nanoparticles:** Fullerenes and carbon nano-tubes (CNTs) represent two major classes of carbon-based NPs. Fullerenes contain nano-material that are made of globular hollow cage such as allotropic forms of carbon. They have created noteworthy commercial interest due to their electrical conductivity, high strength, structure, electron affinity, and versatility (11). These materials possess arranged pentagonal and hexagonal carbon units, while each carbon is sp2 hybridized.

   CNTs are elongated, tubular structure, 1–2 nm in diameter (12). These can be predicted as metallic or semiconducting reliant on their diameter telicity (13). These are structurally resembling to graphite sheet rolling upon itself. The rolled sheets can be single, double or many walls and therefore they named as single-walled (SWNTs), double-walled (DWNTs) or multi-walled carbon nano-tubes (MWNTs), respectively. They are widely synthesized by deposition of carbon precursors especially the atomic carbons, vaporized from graphite by laser or by electric arc on to metal particles. Lately, they have been synthesized via chemical vapor deposition (CVD) technique (14). Due to their unique physical, chemical and mechanical characteristics, these materials are not only used in pristine form but also in nano-composites for many commercial applications such as fillers (15) (16), efficient gas adsorbents for environmental remediation (17), and as support medium for different inorganic and organic catalysts (18).

ii. **Metal NPs:** Metal NPs are purely made of the metals precursors. Due to well-known localized surface plasmon resonance (LSPR) characteristics, these NPs possess unique

optoelectrical properties. NPs of the alkali and noble metals i.e. Cu, Ag and Au have a broad absorption band in the visible zone of the electromagnetic solar spectrum. The facet, size and shape controlled synthesis of metal NPs is important in present day cutting-edge materials (19). Due to their advanced optical properties, metal NPs find applications in many research areas. Gold NPs coating is widely used for the sampling of SEM, to enhance the electronic stream, which helps in obtaining high quality SEM images.

iii. **Ceramics NPs:** Ceramics NPs are inorganic nonmetallic solids, synthesized via heat and successive cooling. They can be found in amorphous, polycrystalline, dense, porous or hollow forms (20). Therefore, these NPs are getting great attention of researchers due to their use in applications such as catalysis, photocatalysis, photodegradation of dyes, and imaging applications (21).

iv. **Semiconductor NPs:** Semiconductor materials possess properties between metals and nonmetals and therefore they found various applications in the literature due to this property (22) (23). Semiconductor NPs possess wide bandgaps and therefore showed significant alteration in their properties with bandgap tuning. Therefore, they are very important materials in photocatalysis, photo optics and electronic devices (24). As an example, variety of semiconductor NPs are found exceptionally efficient in water splitting applications, due to their suitable bandgap and bandedge positions (25).

v. **Polymeric NPs:** These are normally organic based NPs and in the literature a special term polymer nanoparticle (PNP) collective used for it. They are mostly nanospheres or nanocapsular shaped (26). The former are matrix particles whose overall mass is generally solid and the other molecules are adsorbed at the outer boundary of the spherical surface. In the latter case the solid mass is encapsulated within the particle

completely (27). The PNPs are readily functionalize and thus find bundles of applications in the literature (28)(29).

vi. **Lipid-based NPs:** These NPs contain lipid moieties and effectively using in many biomedical applications. Generally, a lipid NP is characteristically spherical with diameter ranging from 10 to 1000 nm. Like polymeric NPs, lipid NPs possess a solid core made of lipid and a matrix contains soluble lipophilic molecules. Surfactants or emulsifiers stabilized the external core of these NPs (30). Lipid nanotechnology is a special field, which focus the designing and synthesis of lipid NPs for various applications such as drug carriers and delivery and RNA release in cancer therapy (31).

1.3 SOLID LIPID NANOPARTICLES:

Lipid based formulations were first commercialized in the 1950s. Intralipid was introduced as a safe fat emulsion for parenteral nutrition followed by Diazemuls, Diazepam-Lipuro and Etomidate-Lipuro which were subsequently introduced into the market. The potential reason behind the popularity of lipid delivery systems is the reduced pain and inflammation at the site of injection (32). A particular advantage offered by lipid colloidal carriers is the increase in bioavailability of poorly water-soluble drugs. Melt-emulsified nanoparticles based on lipids that are solid at room temperature offers several advantages when compared to nano-emulsions, nano-suspensions, mixed micelles, liposomes and polymeric nanoparticles. Even though polymeric nanoparticles have proven to be excellent drug carriers there are few associated limitations such as inclusion of residues from organic solvents used during formulation, toxicity of the polymer, and production scale up.

- **Advantages of Solid Lipid Nanoparticles:**

a) Broad spectrum of route of administration (dermal, intravenous, etc.)

b) Good physical stability.

c) Protection from degradation of incorporated labile drugs.

d) Modulated (fast or sustained) release of the drug.

e) Targeted drug delivery.

f) No use of organic solvents during preparation.

g) Ease of scale up.

h) Excellent biocompatibility.

i) No need of special solvents.

j) Conventional emulsion production techniques can be used.

k) Raw materials same as used in the production of emulsions can be used.

l) Can be sterilized by commercial sterilization methods. (33) (34)

- **Disadvantages of Solid lipid nanoparticles :**

a) Particle growth.

b) Gelation tendency.

c) Unexpected polymorphic transitions.

d) Possibility of metal contamination.

e) High polydispersity.

f) Thermal degradation of heat labile drugs.

g) Presence of super-cooled melts. (35)

1.4 SOLID LIPID NANOPARTICLES PRODUCTION TECHNIQUES:

For several years, solid lipids have been used in the form of pellets to achieve delayed drug release (36). In the early 80s, Speiser and coworkers developed spray dried and congealed micropellets and nanopellets of lipids for oral administration (37). Nanopellets developed by Speiser often contained high amounts of microparticles. Domb produced lipospheres by high shear mixing or ultra sonication. But both the

nanopellets and lipospheres produced by Speiser and Domb respectively were 5 contaminated by microparticles. Since the last decade several scientists have realized the potential of SLNs technology and their research efforts have brought about improvement in solid lipid nanoparticles synthesis.

- **High pressure homogenization:**

Muller and Lucks were the first to prepare solid lipid nanoparticles by applying high pressure homogenization (HPH) technique. Homogenizers have been used commercially for several years now for the production of nanoemulsions for parenteral nutrition, such as Intralipid® and Lipofundin® (38). Thus, scaling up represents fewer problems when compared to other techniques and it is cost effective. Naturally, a lot of research has been done utilizing this method to produce better solid lipid nanoparticles by several research groups. A homogeneous dispersion with narrow size distribution is desirable to increase the physical stability of the aqueous dispersion. In this technique the liquid is forced at a high pressure (100-2000 bar) through a narrow gap of few microns. The resulting high shear stress and cavitation forces decrease the particle size. If the particles localized at different positions in the dispersion volume experience different forces then the degree of particle disruption will vary. The two basic production methods to HPH are the hot and the cold homogenization techniques. In both the techniques, the lipid contents are in the range of 5-10%, but even higher concentration of lipid (40%) can be homogenized to nano-dispersion (39).

- **Hot Homogenization Technique:**

In this method, the drug is first dissolved or solubilized in the lipid being melted at (5-10) ºC above its melting point. The drug-containing melt is dispersed under stirring in a hot aqueous surfactant solution of identical temperature (40). The pre-emulsion is then

passed through a high pressure homogenizer (e.g. Micron LAB40) for 3 to 5 cycles and applying a pressure of about 500-1500 bar (41). The obtained nanoemulsion is cooled down to room temperature or lower. The lipid re-crystallizes and leads to SLNs. Homogenization pressure and the number of cycles should not be higher than that required to achieve the desired effects because this increases the cost of production and the chances of metal contamination as well as in some cases it would result in increase in particle size due to aggregation as a result of the high surface free energy of the particles (36). This technique is performed at a high temperature and thus cannot be used for temperature sensitive drugs. So, to avoid heat-accelerated drug degradation, the length of time for drug exposure should be shortened. This method is also not suitable for hydrophilic drugs because they would partition between the melted lipid and aqueous phase during hot homogenization (42). Either medium scale or large scale production is possible for SLNs by HHT and it is the most extensively technique for the preparation of SLNs.

- **Cold Homogenization Technique:**

In this process the active ingredient is first dissolved in the lipid at a temperature above the melting point of the lipid. The mixture is rapidly cooled with the help of liquid nitrogen or dry ice. Rapid cooling procedure helps in the homogeneous distribution of the active ingredient. This solidified mixture is milled to about 50-100 µm particles using a ball or mortar mill (38). The lipid microparticles obtained by milling are suspended in a surfactant solution to obtain a suspension. This suspension is then passed through a high pressure homogenization at or below room temperature to obtain solid lipid nanoparticles. The cold homogenization technique reduces the chances for temperature induced drug degradation and thus thermo sensitive drugs can be used. Since the solid lipid is milled the complexity arising due to lipid modification can be

avoided (36). Chances of drug distribution into the aqueous phase are limited and hence this method can be used for hydrophilic drugs as well as lipophilic drugs. Lipid nanoparticles prepared via this technique possess a slightly larger particle size and polydispersity when compared to the ones obtained by hot homogenization technique, using the same lipid at similar homogenization parameters (pressure, temperature and the number of cycles). A higher number of homogenization cycles can be applied to reduce the particle size (43).

- **Microemulsion based technique:**

Gasco and co-workers were the first to develop SLNs based on the dilution of microemulsions (44). Microemulsions are thermodynamically stable, clear and isotropic mixtures usually composed of an oil or lipid, emulsifier and/or co-emulsifier and water. In this process, the solid lipids are melted above their melting point. Separately, a mixture of emulsifier, co-emulsifier and water is heated at same temperature. Both the lipid and the aqueous phase containing the emulsifier are mixed in appropriate ratios and stirred in order to produce a microemulsion. The hot microemulsion is then diluted with cold water (2-8) °C while stirring. The ratio of the hot microemulsion to cold water is usually in the range of 1:10 to 1:50. It has been noted that a droplet structure is already present in the microemulsion and therefore, no external energy is required to achieve the small particle size. When the microemulsion is diluted by cold water, the lipid droplets solidify as the temperature decreases. The temperature gradient and pH value determine the quality of the particles in addition to the composition of the microemulsion. Subsequently, the produced SLNs are washed three times with distilled water and followed by membrane filtration in order to remove any unwanted bigger lipid particle. Due to the dilution of microemulsion the concentrations of particle content are below 1% and, therefore, large amount of water has to be removed either by

ultra-filtration or by lyophilisation in order to increase the particle concentration. The major limitation of this technique is its sensitivity to minor changes in composition or thermodynamic variables, which can lead to phase transitions. Lack of robustness of the microemulsion technique can lead to high production costs. Moreover, lipids solidification shifts the system to a thermodynamically unstable state. The high concentration of surfactants used may produce toxicity. Ultracentrifugation, ultra-filtration or dialysis can be applied to remove excess surfactants (32).

- **Solvent emulsification-evaporation technique:**

Sjöström and Bergenståhl were the first to describe the production of SLNs by solvent emulsification-evaporation technique (45). The solid lipid is dissolved in a water immiscible organic solvent (e.g. cyclohexane, dichloromethane, toluene, chloroform, etc.) and the drug is dissolved or dispersed in the solution (46). This organic phase containing the drug is emulsified in an aqueous surfactant solution by mechanical stirring. The organic solvent is then removed from the emulsion under mechanical stirring or reduced pressure (40-60 mbar) (e.g. rotary evaporator) (46). Lipid nanoparticle dispersion is formed by the precipitation of the lipid phase in the aqueous surfactant medium. The mean particle size depends on the concentration of lipid in organic phase. Very small particle size can be obtained with low lipid load (5%) related to organic solvent. Aggregation of the particles can be avoided by removing the solvent at a faster rate (42). Thermo labile drugs can be incorporated via this technique as it avoids thermal stress. Trace amounts of organic solvent remaining in the final product can potentially create toxicity problems. A large quantity of water has also to be removed during the final step of the formulation by means of ultra-filtration or evaporation (47) (48).

- **Solvent displacement technique:**

Fessi et al. were the first to describe this technique for the preparation of polymeric nanoparticles by polymerization in solution (49). Recently, this technique has been modified and used for the preparation of SLN's (50) (51). In this technique, lipid and the active ingredient is dissolved in a water miscible solvent such as ethanol, isopropanol, acetone, or methanol (52). The mixture is then dispersed into an aqueous solution of a surfactant with mild mechanical stirring producing a suspension of lipid nanoparticles (50). The solvent is subsequently removed by distillation. Ultracentrifugation, ultra-filtration or lyophilization can be used for isolating the lipid nanoparticles.

- **Solvent Emulsification-diffusion technique:**

Quintanar-Guerrero et al. were the first to describe this technique for the preparation of polymeric nanoparticles (53). Recently this technique has been modified by various research groups for the preparation of lipid nanoparticles (54) (55). In solvent emulsification diffusion technique, the lipid is dissolved in a partially water miscible solvent (e.g. benzyl alcohol, isobutyric acid, or tetrahydrofuran) which is previously saturated with water at room temperature or at a controlled temperature in order to ensure the initial thermodynamic equilibrium (32). The mixture is then emulsified in an aqueous solution of a surfactant by mechanical stirring at the temperature used to dissolve the lipid producing an o/w emulsion. This o/w emulsion is then diluted with excess water, in typical ratio ranges from 1:5 to 1:10 (32), at a controlled temperature which causes the diffusion of the solvent into the external phase and subsequent precipitation of the lipid nanoparticles. The diffused solvent can be removed either by vacuum distillation or ultra-filtration. The concentration and the nature of the lipid and

surfactant, stirring rate and the processing temperature are critical variables in this technique. This technique avoids the necessity to melt the lipid which is very useful for preparation of protein and peptide loaded lipid nanoparticles (56).

- **Ultrasonication Technique:**

In this technique, solid lipids are melted at (5–10) °C above their melting points and drug is dissolved or dispersed in melted lipids. Then a hot aqueous surfactant solution (preheated at the same temperature) is added to the drug-lipid melt and homogeneously dispersed by a high shear mixing device. The coarse hot o/w emulsion obtained is ultrasonicated using a probe sonicator till the desired sized nanoemulsion is formed. Finally, lipid nanoparticles are obtained by allowing hot nanoemulsion to cool down to room temperature.

Two mechanisms are proposed for ultrasonic emulsification. First, the application of an acoustic field produces interfacial waves resulting in the dispersion of the oil phase in the continuous phase in the form of droplets (57). Secondly, the application of ultrasound induces acoustic cavitation causing the formation and subsequent collapse of micro bubbles by the pressure fluctuations of a simple sound wave, which creates extreme levels of highly localized turbulence. Therefore, the turbulent micro-implosions break up primary droplets into sub-micron size (58).

This technique is a simple and reproducible way to prepared nanoparticles without the need of organic solvents or any sophisticated instruments and has the potential to easily scale up for large scale production. However, metallic contamination of the product may occur during sonication by probe sonicator (59).

- **Double Emulsion Technique:**

The double emulsion (w/o/w) method is based on solvent emulsification–evaporation method (32). This method is mainly for the production of lipid nanoparticles loaded with hydrophilic drugs. In this case, drug is firstly dissolved in aqueous solvent (inner aqueous phase) and then is dispersed in lipid containing emulsifier/stabilizer to produce primary emulsion (w/o). A double emulsion (w/o/w) is formed after addition of an aqueous solution with a hydrophilic emulsifier followed by stirring. Each emulsification step results in a highly polydisperse droplet distribution, exacerbating the polydispersity of the final double emulsion. Thus, lipid nanoparticles formed from double emulsions technique are, by nature, poorly controlled in both size and structure (60).

- **Supercritical fluid method:**

This is a novel technique recently applied for the production of SLNs. A fluid is termed supercritical when its pressure and temperature exceed their respective critical value. The ability of the fluid to dissolve compounds increases. This technology comprises of several processes for nanoparticle production such as rapid expansion of supercritical solution (RESS), particles from gas saturated solution (PGSS), aerosol solvent extraction solvent (ASES), supercritical fluid extraction of emulsions (SFEE). The advantages of this technique includes avoidance of the use of solvents, particles obtained as a dry powder, instead of suspensions, requires mild pressure and temperature conditions. Carbon dioxide solution is the good choice as a solvent for this method (40).

- **Spray drying method:**

It is an alternative technique to the lyophilization process. This recommends the use of lipid with melting point more than 70 °C. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture (61).

- **Precipitation method:**

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles (40).

1.5 DRUG LOADING CAPACITY OF SOLID LIPID NANOPARTICLES:

Drug loading capacity is an important parameter to investigate the suitability of a drug carrier system. The drug loading capacity is related to the nature of the lipid and is generally expressed in terms of percentage. The drug loading capacity varies depending on the drug incorporated in the SLNs. Drug loading capacities ranging from 1% to 50% have been reported. 5- Florouracil was found to be encapsulated 46% in SLNs prepared by novel temperature modulated solidification technique (62).

Factors that determine the drug loading capacity of SLNs are as follows:

a) Drug solubility in the lipid melt.
b) Drug melt and lipid melt miscibility.
c) Structure of the lipid matrix
d) Polymorphic state of the lipid (42).

However, it has been observed that the drug loading capacities of lipid nano-carriers are comparatively lower due to low solubilization capacities of the molten lipids for the

hydrophobic drug entities. Super-cooled melts have found to have higher drug-loading capacities when compared to crystallized nanoparticles. Hard fats have higher drug loading capacities due to their crystalline nature when compared to pure monoacid triglycerides (63).

The drug solubility reduces when the lipid melt is cooled down. Hence, the solubility of the drug in the lipid should be higher than required. The drug solubility is enhanced in the presence of mono- or di-glycerides and also by addition of solubilizers. A good drug loading capacity is obtained when polydisperse lipids that are generally used in cosmetics, are used in SLNs (64).

The chemical nature of the lipid is a key-determining factor of drug loading capacity in SLNs. Determination of crystallinity of the lipids and other excipients may prove to be beneficial for predicting the drug entrapment. Optimized drug incorporation and physical characterization of the lipids and other excipients using analytical techniques such as nuclear magnetic resonance, powder X-ray diffraction, differential scanning calorimetry, fourier transform infrared spectroscopy, etc. are required (42).

The polymorphic form of the lipid is a major determinant of the drug loading capacity. The polymorphic form in which a lipid is present differs depending on whether it is in the bulk form or if it is in the form of nanoparticles. It has been found that the lipid, when present as nanoparticles, re-crystallizes at least partially if not fully into α-form. In contrast, the bulk lipid is found to re-crystallize into β-form. The formation of the β-form results in expulsion of the drug from the lipid matrix. This transformation is slower for long-chain triglycerides than short chain triglycerides (65). Dispersed lipids are found to re-crystallize in α-form whereas the bulk lipids are found to re-crystallize

in β′-modification followed by transformation into the β-form if heated above the bulk melting temperature and then cooling under controlled conditions.

SLNs may be optimized in a way to produce and maintain some fraction of α-form to achieve better entrapment and controlled release of the drug (64).

1.6 DRUG RELEASE FROM SOLID LIPID NANOPARTICLES:

The release of the entrapped drug from the SLNs is governed by the following principles,

a) An inverse relationship exists between the release of the drug and the partition coefficient of the drug.
b) Smaller particle size promotes higher surface area thereby leading to higher drug release.
c) Homogeneous dispersion of the drug in the lipid matrix causes slow release of the drug.
d) Lipid crystalline and high drug mobility leads to rapid release of the drug from the SLNs (66).

Numerous studies have been carried out on the effect of formulation parameters and process conditions on the release of drug from the SLNs.

SLNs incorporated with tetracaine and etomidate demonstrated burst release with 100% release of the drug in less than 1 minute. This release pattern was attributed to the large surface area of nanoparticles and higher percentage of drug in the outer layer of the nanoparticles. However, a prolonged drug release pattern was observed for lipid soluble prednisolone loaded SLNs. Hence, it was concluded that SLNs incorporated with lipophilic drugs follows prolonged release patterns (67).

In another investigation, Jenning et al. compared the drug release pattern of the SLNs with the drug release pattern of nanoemulsions (68). Retinol was incorporated into the SLNs and nanoemulsions. Retinol is highly unstable in water and degrades within few days at room temperature. As the vitamin A has very low aqueous solubility even in the presence of surfactants, it was assumed that the detected amount of vitamin A was present in the lipid phase of the SLNs or Nano-emulsion. They found two different types of drug release profiles for SLNs and nanoemulsions. For the initial 6 h only one-third fraction of the drug was released from the SLNs compared to that released from nanoemulsion. However, for the fractions collected after 18 and 24 h, the drug release rate of SLNs increased and even exceeded the release rate of nanoemulsion which was attributed to the evaporation of water from SLNs during the experiment and subsequent formation of gel leading to faster release of drug (68).

The parameters that affect the release of drug from SLNs are temperature, amount of drug incorporated, lipid structure, drug structure, duration of production, processing equipment, lyophilization process, sterilization process (66)

Among these, the two major parameters that influence the release of drug from the SLNs are temperature and presence of a surfactant.

- **Influence of Temperature on Release of Drug from SLNs:**

The release profile of SLNs generally follows a biphasic pattern. A burst release is observed initially followed by a prolonged release. Drug release investigations have proven that highest burst release is observed at highest temperatures of production and also if hot homogenization is used as the method of production. Also, burst release is found to decrease with decreasing production temperature and is negligible with cold homogenization technique. The use of high temperature facilitates solubility of drug in

the aqueous phase. Hence, use of lower production temperatures may eliminate burst release of the drug (66) (67).

- **Influence of Surfactants on Release of Drug from SLNs:**

The amount of surfactant or surfactant mixture in the formulation also influences the burst release of the drug from SLNs. It has been found that at high surfactant concentrations the burst release of the drug is higher, while at lower concentrations of surfactants or surfactant mixtures the burst release is lower. This phenomenon is supported by the hot homogenization process in which redistribution of the drug occurs between the lipid and the aqueous phase during the heating process followed by a subsequent cooling process. As the dispersion of lipid and water is heated, the drug travels from melted lipid droplet to the aqueous phase. Then as the oil/water emulsion is being cooled, the solubility of the drug in the water continuously decreases with a decrease in the temperature leading to repartitioning of the drug into the lipid phase. A formation of the solid lipid core including the drug starts at the re-crystallization temperature of the lipid. As the temperature further decreases the pressure on the drug increases to repartition into the lipid phase because of the decrease in the solubility of the drug in water. But, as the lipid core is crystallized, the drug cannot incorporate into the lipid core (42). This leads to super saturation of the aqueous phase with the drug and results in formation of outer liquid layer of SLN enriched with the drug. Therefore, hydrophilic drugs are observed to have higher burst release. Use of surfactants facilitates dissolution of drug in the aqueous phase. Hence, SLNs having low surfactant concentration or no surfactant may eliminate burst release (66) (67).

- **Controlled Release of Actives from SLNs:**

It has been found that there is a reduction in burst release with an increase in the particle size of the SLNs. Also, with a little larger particle sizes, prolonged release could be obtained (i.e. micro-particles). Homogenization process has a considerable influence on the release of drug. The drug release profiles may be altered depending on the production technique (i.e. hot/cold homogenization) of SLNs and type of lipid used. The sustained release of drug from SLNs may be explained based on the concept of molecular distribution of the drug in the lipid according to the solid solution model. The elimination of burst release with cold-homogenization process may be attributed to the homogeneous molecular distribution of the drug in the solid lipid matrix which results in the formation of a solid dispersion prior to homogenization and particle formation process. Because the SLNs are solids at room temperature, the mobility of drug is highly reduced thereby leading to controlled release of drug (69).

1.7 CHARACTERIZATION OF SOLID LIPID NANOPARTICLES:

Characterization of solid lipid nanoparticles is a challenge due to the small size of these colloidal carriers and complexity of the system. Numerous parameters need to be considered, such as mean particle size, zeta potential, drug association efficiency, morphology and in-vitro study. For each parameter can be applied several techniques.

- **Measurement of Particle Size:**

Particle size might be determined by dynamic light scattering (DLS), optical single particle sizing (OSPS), laser diffraction (LD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunnelling microscopy (STM) and freeze fracture electron microscopy (FFEM) (36).

- **Dynamic Light Scattering:**

Dynamic Light Scattering (DLS) also referred as Photo Correlation Spectroscopy (PCS) is a technique used to determine the mean particle size and the width of the particle size distribution expressed as polydispersity index (PDI). The particle size is the diameter of the sphere that diffuses at the same speed as the particle being measured.

The measurement using DLS is based on the light scattering phenomena in which the statistical intensity fluctuations of the scattered light from the particles in the measuring cell are measured. These fluctuations are due to the random movement of the particles in the dispersion medium. This movement is called Brownian motion (32).

Usually a DLS device consists of a laser light which illuminates a small volume of the sample composed by a dilute suspension of particles. The light scattered from these particles is collected by a lens and its intensity is measured by a photomultiplier at a certain angle (90º or 173º). The diffusion rate of the particles depends on their size (at a known fluid viscosity and temperature). Hence, the size of these particles can be calculated from the rate of fluctuation of the scattered light intensity. When the suspended particles are small, they diffuse relatively fast, and the fluctuations in the scattered light are rapid. On the other hand, if the particles are large, they move slowly, and the fluctuations in the scattered light are slow. The detected intensity signals are used by a correlator to calculate the auto-correlation function $G(\tau)$. A correlator is basically a signal comparator designed to measure the degree of similarity between two signals, or one signal with itself at varying time intervals. From the decay of correlation function, the diffusion coefficient (D) of the particles is obtained. Once the diffusion coefficient is known, the hydrodynamic diameter of a spherical particle can be calculated applying the Stokes-Einstein equation which is given below,

$$d(H) = \frac{KT}{3\pi\eta D}$$

where, d(H) is the hydrodynamic diameter, D is translational diffusion coefficient which measures the velocity of the Brownian motion, k is the Boltzmann's constant, T is the absolute temperature and η is the viscosity of the solution. As it was previously mentioned, small particles diffuse faster than large ones, causing a stronger fluctuation in the scattering signal and a more rapid decaying G(τ) (Figure 1.1). For a monodisperse particle population, G(τ) is a single exponential, but if more than one size of particles is present the function is poly-exponential. Deviation from a single exponential is used to calculate the PDI, which is a measure of the width of the size distribution. The PDI value is zero when a monodisperse particles population is measured. PDI values of around 0.10-0.30 indicate a relatively narrow distribution, values of 0.5 and higher are obtained in case of very broad distributions (70).

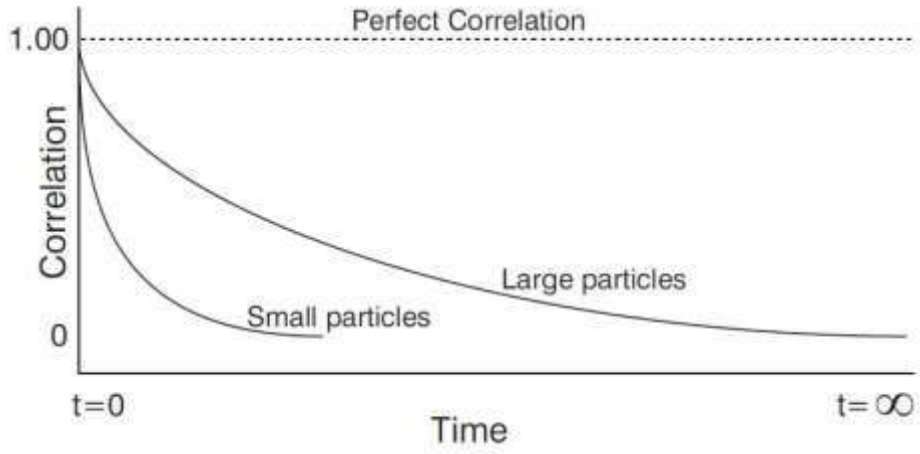

Figure 1.1 Correlation differences between small and large particles. From (70).

- **Encapsulation Efficiency:**

According to literature, the association efficiency of a drug in SLNs is most frequently assessed indirectly, meaning that the aqueous phase is separated from the lipid particles and subsequently drug content of the aqueous phase is determined (32). The separation

of the SLNs from the aqueous phase can be done by e.g. ultra-filtration, ultracentrifugation and gel separation. The amount of drug measured in the aqueous phase is considered the amount of drug not incorporated. The following equation is used to calculate the association efficiency:

$$\text{EE\%} = \frac{\text{TD} - \text{F}D}{\text{TD}} \times 100$$

Where TD is total drug weighted at the beginning and FD is the free drug dissolved in aqueous medium.

- **Morphology:**

Images of lipid nanoparticles could be achieved through several microscopic techniques, such as the scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), CryoSEM and CryoTEM (36). These techniques are very useful since allow the visualization and measurement of particles much smaller than 1 μm.

- **Measurement of Zeta Potential:**

Almost all particles in contact with a liquid acquire an electric charge on their surface. The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions close to the surface. Thus an electrical double layer exists around each particle (Figure 1.2). The liquid layer surrounding the particle has an inner region called the Stern layer, where the ions are strongly bound and an outer and diffuse region, where the ions are less firmly attached. Within the diffuse layer there is an imaginary boundary inside which the ions and particles form a stable entity. When a particle moves, ions within the boundary move with it, but any ions beyond the boundary do not move with

the particle. This boundary is called the surface of hydrodynamic shear or slipping plane. Zeta potential (ζ) is the potential that exists at this boundary and it is a parameter which is very useful for the assessment of the physical stability of colloidal dispersions (70).

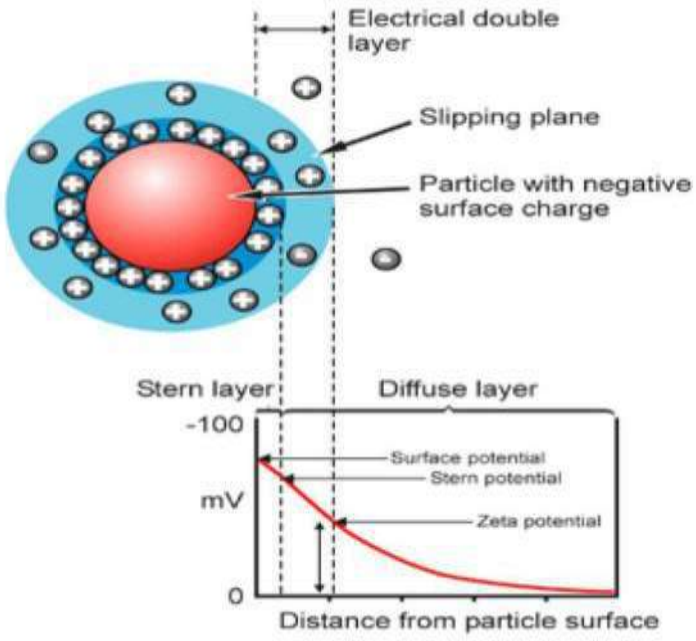

Figure 1.2 Zeta-potential of a nanoparticle in solution. (70).

Charged particles suspended in the electrolyte are attracted towards the electrode of opposite charge, when an electric field is applied. Viscous forces acting on the particles tend to oppose this movement. When equilibrium between these two opposing forces is achieved, the particles move with a constant velocity. This velocity of the particle per unit electric field strength (E) is called the electrophoretic mobility (μ), which is expressed in micrometers per second per volt per centimeter (μm/s)/(V/cm). Zeta Potential can be measured by electrophoretic mobility through Henry's equation,

$$\mu = \frac{2\varepsilon\zeta f(ka)}{3\eta}$$

Where η is the viscosity of the dispersion medium, ε the permittivity of the environment (the dielectric constant) and f(ka) is the Henry's function. Electrophoretic determinations of zeta potential are most commonly made in aqueous media and moderate electrolyte concentration. In this case, f(Ka) is 1.5 and is referred to as the Smoluchowski approximation. Non-aqueous measurements generally use the Huckel approximation and f(Ka) is 1 (70).

The storage stability of a colloid system can be predicted by the measurement of the zeta potential. It is currently accepted that absolute zeta potentials values higher than 30 mV are required for full electrostatic stabilization; potentials between 5 and 15 mV are in the region of limited flocculation; and lower than 3 mV of maximum flocculation. As a result, particle aggregation is less expected to occur for charged particles (high zeta potential) due to electric repulsion (32).

Nanoparticles with a zeta potential between -10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively. Since most cellular membranes are negatively charged, zeta potential can affect a nanoparticle's tendency to permeate membranes, with cationic particles generally displaying more toxicity associated with cell wall disruption. Positive charge possesses a membrane destabilizing and concomitantly destructive effect resulting from an interaction of positive charge and negative charge of membrane. Thus, positively charged lipids are not approved by Food and Drug Administration (FDA) for clinical use (71).

- **Transmission Electron Microscopy:**

TEM utilizes energetic electrons to provide morphologic, compositional and crystallographic information of samples. At a maximum potential magnification of 1 nm, TEM is the most powerful microscope, producing high-resolution and two-dimensional images, allowing the investigation of micro- and nanostructures. Going through the several parts of the TEM system, first an electron gun generates a high energy electron beam by accelerating electrons emitted from a cathode. This acceleration voltage, usually greater than 100 kV, determines the microscope's resolution. After the accelerator, there are condenser lenses to control beam diameter and convergence angles of the incident beam on a specimen. The TEM has three lenses to ensure good magnification capability, and the intermediate lens is used to switch the TEM between an image mode and a diffraction mode. TEM specimens must be thin foils (about 100 nm) because they should be able to transmit electrons, and this thin specimen is mounted in a 3 mm diameter grid. The specimen preparation depends on the type of sample (36).

- ***In vitro* drug release:**

Dialysis tubing: *In vitro* drug release could be achieved using dialysis tubing. The solid lipid nanoparticle dispersion is placed in pre - washed dialysis tubing which can be hermetically sealed. The dialysis sac then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the dissolution medium at suitable intervals, centrifuged and analyzed for the drug content using a suitable analytical method (35).

Reverse dialysis: In this technique a number of small dialysis sacs containing 1 ml of dissolution medium are placed in SLN dispersion. The SLN's are then displaced into the medium (72).

1.8 APPLICATIONS OF SLNS IN DRUG DELIVERY SYSTEM:

- **SLNs as gene vector carrier:**

Cationic solid lipid nanoparticles have established themselves during the past decades. They can well bind DNA directly via ionic interaction and intervene gene transfection. SLN can be used in the gene vector formulation. There are several recent reports of SLN carrying genetic/peptide materials such as DNA, plasmid DNA and other nucleic acids. Cationic solid lipid nanoparticles are promising non viral gene delivery carriers suitable for systemic administration. The relationship between the composition of cationic SLN and their ability to condense plasmid DNA (pDNA) and to transfer it in neuroblastoma cells were investigated. The lipid nucliec acid nanoparticles were prepared from a liquid nanophase containing water and a water miscible organic solvent where both lipid and DNA are separately dissolved by removing the organic solvent, stable and homogeneously sized lipid-nuclic acid nanoparticle (70-100 nm) were formed. It's called genospheres. Mannanmodified DNA-loaded vehicles have great potential for targeted gene delivery (36).

- **SLNs as a targeted carrier for solid tumors:**

One of the most important challenges in drug delivery is to get the drug at the place it is needed in the body thereby avoiding possible side effects to non diseased organs. The non restricted toxicity of chemotherapeutics thus limits the full use of their therapeutic potential. Local drug delivery or drug targeting results in increased local drug concentrations and provides strategies for more specific therapy. Nanoparticles have specific particles as tools to enable these strategies. SLNs have been reported to be

useful as drug carriers to treat neoplasms. Tumour targeting has been achieved with SLNs loaded with drugs like methotrexate, paclitaxel and camptothecin (73).

- **SLNs in anti tubercular chemotherapy:**

Another prominent example of SLNs-based drug delivery is pulmonary delivery of antimicrobials to treat tuberculosis, a serious lung infection caused by Mycobacterium tuberculosis. Antitubercular drugs such as rifampicin, isoniazide, pyrazinamide-loaded SLN systems, were able to decrease the dosing frequency and improve patient compliance. This antitubercular drug loaded solid lipid nanoparticles were prepared by using the emulsion solvent diffusion technique (42).

- **SLNs in breast cancer:**

Photodegradation and low bioavailability are chief hurdles for the therapeutic use of curcumin. Transferrin mediated SLNs were formulated to increase photostability and enhance its anticancer activity against MCF-7 breast cancer cells. The anticancer activity of curcumin is enhanced with transferrin-mediated SLNs compared to curcumin solubilized surfactant solution and apoptosis is the mechanism underlying the cytotoxicity. Mitoxantrone-loaded SLNs local injections were formulated to reduce the toxicity and improve the safety and bioavailability of drug. Efficacy of doxorubicin has been reported to be enhanced by incorporation in SLNs. Doxorubicin was complexed with soybean-oil-based anionic polymer and dispersed collectively with a lipid in water to form doxorubicin loaded solid lipid nanoparticles. The system has improved its efficacy and reduced breast cancer cells (74).

- **SLNs for topical use:**

Corticosteroids are therapeutic agents generally used in the treatment of skin diseases such as eczema or psoriasis. Topical SLN products show enormous prospective for treating dermatological conditions by targeting corticosteroids to dermal disease sites

while decreasing systemic drug absorption. Topical application of the drugs at the pathological sites offers possible advantages of delivering the drug directly to the site of action. SLNs are used for topical application of various drugs such as vitamin-A, isotretinoin, flurbiprofen. The isotretinoin-loaded lipid nanoparticles were formulated for topical delivery of drug. Production of the flurbiprofen-loaded SLN gel for topical application offer a potential advantage of delivering the drug directly to the site of action, which will produce higher tissue concentrations. Miconazole nitrate loaded SLN were prepared by modified solvent injection method and characterized for surface morphology, particle size and drug entrapment (75).

- **SLNs as cosmeceuticals:**

Cosmeceuticals is rising as the major application target of these carriers. Carrier systems like SLNs and NLC were formulated with a point of view to meet manufacturing needs like scale up, qualification and validation, simple technology, low cost etc. The SLNs have been functional in the preparation of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers. Many features of SLNs are advantageous for dermal application of cosmetic products have been reported, e.g. occlusive properties, increase in skin hydration, modified release, increase of skin penetration and avoidance of systemic uptake. The first two cosmetic products containing lipid nanoparticles were introduced to the market in 2005. Within 3 years after the introduction, of about 30 cosmetic products containing lipid nanoarticles are in the market these days (36).

- **Solid lipid nanoparticles for delivering peptides and proteins:**

Solid lipid particulate systems such as solid lipid nanoparticles (SLN), lipid microparticles (LM) and lipospheres have been sought as alternative carriers for therapeutic peptides, proteins and antigens. The research work developed in the area

confirms that under optimized conditions they can be produced to incorporate hydrophobic or hydrophilic proteins and seem to fulfill the requirements for an optimum particulate carrier system. Proteins and antigens intended for therapeutic purposes may be incorporated or adsorbed onto SLN, and further administered by parenteral routes or by alternative routes such as oral, nasal and pulmonary. Formulation in SLN confers improved protein stability, avoids proteolytic degradation, as well as sustained release of the incorporated molecules. Important peptides such as cyclosporine A, insulin, calcitonin and somatostatin have been incorporated into solid lipid particles and are currently under investigation. Several local or systemic therapeutic applications may be foreseen, such as immunisation with protein antigens, infectious disease treatment, chronic diseases and cancer therapy (76).

- **SLN as potential new adjuvant for vaccines:**

Adjuvants are used in vaccination to enhance the immune response. The safer new subunit vaccines are less effective in immunization and therefore effective adjuvants are required. New developments in the adjuvant area are the emulsion systems. These are oil-in-water emulsions that degrade rapidly in the body. Being in the solid state, the lipid components of SLNs will be degraded more slowly providing a longer lasting exposure to the immune system (73).

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CHAPTER - 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 LITERATURE REVIEW

Scott *et al., (2008*) The absolute bioavailability of olmesartan following a single 20 mg oral dose in healthy volunteers was 26% and peak plasma levels were attained after approximately 2 hours. Olmesartan is highly bound to plasma proteins and has a low volume of distribution. It is not further metabolized and is excreted mainly in the faeces, via the hepato-biliary route, with smaller amounts in the urine. The long elimination half-life permits once-daily administration. Olmesartan seldom requires dosage adjustment and has a low potential for pharmacokinetic drug-drug interactions (1).

Gupta *et al., (2009)* Drugs with poor solubility possess difficulty in formulation by applying conventional approaches as they show problems like slow onset of action, poor oral bioavailability, lack of dose proportionality, failure to realize steady-state plasma concentration, and undesirable side effects. The traditional dosage forms thus may end in over-or under medication and poor patient compliance. These types of challenges can be overcome by applying novel drug delivery systems, offering benefits such as reduction in dose frequency, enhanced permeability, lowering of dose size, site-specific targeting, and improvement in oral bioavailability (2)

Suri *et al., (2007)* Nanotechnology is a promising strategy with the advancement in drug delivery systems especially for those potent drugs whose clinical development failed; credit to their poor solubility, inadequate bioavailability, low permeability, and other poor biopharmaceutical properties. The foremost common nanotechnology-based strategies utilized in the development of delivery systems are nanoemulsions, dendrimers, micelles, liposomes, solid lipid nanoparticles, polymeric nanoparticles, carbon nanotubes, and so forth, which give controlled, sustained, and targeted drug

delivery. The nanotechnology-based systems have extensively been investigated for improvement of the bioavailability of antihypertensive drugs (3)

Attari *et al., (2015)* Prepared and evaluated Olmesartan loaded nanosuspensions by combinative technologies viz. ball milling followed by probe sonication and high speed homogenization followed by probe sonication stabilizers such as PVA and Poloxamer 188. The prepared nanosuspension showed the optimum particle size 469.9 nm and zeta potential −19.1 mV. In ex vivo intestinal absorption, the absorption and permeability of OLM nanosuspension were observed to be increased as compared to pure drug. This shows that there was better reduction in particle size resulting in increased surface area which increased the permeation and eventually increased the absorption (4)

Chowdary KPR *et al., (2014)* Prepared and evaluated solid dispersions of Olmesartan in crospovidone and poloxamer 188 alone and in combination of both for enhancing the dissolution rate and dissolution efficiency of Olmesartan. Solvent evaporation method was used to prepare solid dispersion of Olmesartan. Solid dispersions of Olmesartan in crosspovidone alone were prepared using five ratios of drug: carrier namely 9:1, 8:2, 2:1, 1:1 and 1:3 and in Poloxamer 188 alone were prepared using three ratios of drug: carrier namely 19:1, 9:1 and 8: 2 by common evaporation solvent method. All the solid dispersions prepared were evaluated for drug content uniformity, dissolution rate and dissolution efficiency in comparison to Olmesartan pure drug. Solid dispersions prepared employing crosspovidone and poloxamer 188 alone as carriers gave rapid and higher dissolution of Olmesartan. With the both carriers, the dissolution rate and dissolution efficiency of Olmesartan were increased as the percent of carrier in the solid dispersion was increased. (5)

Nagaraj *et al., (2017)* Prepared nanosuspensions of Olmesartan by high pressure homogenization method using Poloxamer-188, PVP k30 and tween 80. In this study, optimized OM-NS was developed by Box Behnken design and characterized. Statistically optimized OM-NS formulation exhibited mean particle size of 492 nm, ZP of -27.9 mV and 99.29% release in 5 min. OM-NS showed more than 4 times increase in its solubility than pure Olmesartan. The pharmacokinetics studies in rats of lyophilized optimized nanosuspensions showed 2.45 and 2.25 folds improvement in oral bioavailability when compared with coarse suspension and marketed tablet powder suspension, respectively. Hence, these results confirmed the potential of NS as suitable delivery system for oral delivery of Olmesartan in improving the oral bioavailability (6)

Mahajan *et al., (2017)* Telmisartan loaded SLN were prepared using stearic acid as matrix forming lipid and Poloxamer 188 as stabilizing emulsifier by the Modified emulsification–ultrasonication technique. The mean particle size of SLN measured to be 391.7nm with PDI value of 0.324, and zeta potential value of 21.5±0.4 mV was observed. The entrapment efficiency was estimated to be 97.58 % for the optimized formulation. The drug was successfully incorporated with high entrapment efficiency of 97.58% for the optimized formulation. The in vivo and in vitro results have assured that SLN of Telmisartan could be successful drug delivery option to enhance its efficacy to treat hypertension. The pharmacokinetic results indicated that the oral bioavailability of Telmisartan was significantly improved after its incorporation into SLN (7)

Naseri *et al., (2015)* In the early days of the 20 century, Paul Ehrlich envisioned his magic bullet concept; the idea that drugs reaches the right site in the body, at the right time, at right concentration. It should not exert side effects, neither on its way to the therapeutic target, nor at the target site, nor during the clearance process. The SLNs have the potential to achieve, at least partially, these broad objectives. Apart from these,

the regular objective of controlled drug delivery is aptly achieved with SLNs. SLNs are developed to overcome the limitations of other colloidal carriers, such as emulsions, liposomes and polymeric nanoparticles because they have advantages like good release profile and targeted drug delivery with excellent physical stability (8)

Nooli *et al., (2016)* Olmesartan loaded solid lipid nanoparticles were prepared by solvent emulsion evaporation method using Tween 80, Poloxamer 188, glyceryl monostearate (GMS) and soya phosphatidylcholine. It showed an average particle size of 100 nm, PDI 0.291, zeta potential of -23.4mV and 78% entrapment efficiency. Pharmacokinetic evaluation in male Sprague Dawley rats revealed 2.32-fold enhancement in relative bioavailability of drug from SLN when compared to that of OLM plain drug on oral administration (9)

Okorie *et al., (2017)* Optimized olmesartan medoxomil loaded solid lipid nanoparticles was prepared by hot homogenization and ultra-sonication method using glyceryl mono stearate, soya lecithin, polysorbate 80. They possess particle size range of 122.8-135.0 nm, and polydispersity index range of 0.205-.206. The particle size results suggest that the formulations have the potential to give high drug release from the nanoparticles matrix and good gastrointestinal uptake of the drug. It has been reported that particle sizes less than 300 nm are advisable for the intestinal transport and polydispersity index value less than 0.3 is often accepted as optimum value. The formulations were found to enhance the bioavailability of olmesartan medoxomil by 2.2 and 2.5 folds respectively, with reference to the oral tablet. The drug was found to be efficiently entrapped in the lipid matrix. The pharmacokinetic studies in vivo showed the solid lipid nanoparticles formulations improved the bioavailability of olmesartan medoxomil (10)

Pandya et al., (2017) Olmesartan loaded SLNs were prepared using hot homogenization method with Glyceryl monostearate (Imwitor 491/GMS) as lipid and Pluronic P407: Tween 80 (1:1 w/w) as surfactant at concentration 1.84%. The results showed that the OLM loaded SLNs were found to be stable; nanometer sized spherical particles (113.10 ± 8.53nm), with low PDI (0.189 ± 1.52) and better in vitro release in stimulated fluids. In vivo pharmacokinetic study of olmesartan loaded solid lipid nanoparticles revealed higher Cmax of 1610 ng/mL, higher AUC of 15492.50 ng/mL and increased relative bioavailability by almost 2.3 folds compared to marketed formulation. These results clearly indicate that Olmesartan loaded solid lipid nanoparticles are shown to have enhanced bioavailability and effective therapeutic result and thus would be an excellent way to treat hypertension (11)

Barman *et al., (2014)* The stable Nifedipine -SLNs were prepared using two phospholipids, hydrogenated soybean phosphatidylcholine and dipalmitoylphosphatidylglycerol mixed with 2.5% w/v trehalose as a cryoprotectant followed by lyophilization. They employed various grades of two types of silica, such as fumed and precipitated. Silica improved the poor flow property of NI-SLNs to good category as per USP-29. In addition, most of the silica nanocomposites showed the satisfactory results in their physicochemical properties such as particle size, polydispersity index, zeta potential, and recovered potency by around 100 nm, 0.3, −50 mV, and 80%, respectively. Furthermore, it was found that NI-SLNs were easily released form nanocomposites within 30 min, therefore, suggesting an improvement of drug dissolutions (12)

Chandana *et al., (2011)* Valsartan loaded SLNs were prepared by hot homogenization method using Hydrogenated phosphatidyl choline, Tween 20 and 80. The mean particles size, Polydispersity index (PDI) and entrapment efficiency of

optimized formulation was found to be 136.5 nm, 0.424, 80.98% respectively. It was reported that increase in concentration of lipids in combination with surfactant in formulation shows increase in entrapment efficiency and lower particles size. When compared the release of plain Valsartan and that from the Valsartan loaded SLN at 60 min, the SLN showed a sustained type of drug release compared to plain Valsartan. From the above study it could be concluded that SLNs are good drug delivery system to overcome the limitations of other colloidal carriers, such as emulsions, liposomes and polymeric nanoparticles (13)

Ekambaram *et al., (2011)* Ramipril loaded SLN were prepared by hot homogenization followed by the ultrasonication method using Poloxamer 188, Glyceryl monostearate, Glyceryl monooleate, Polysorbate 80, Sorbitan monolaurate and Chloroform and Methanol. The result showed that the particle size was found between the ranges from 104nm to 334 nm for different formulation with different concentration. The entrapment efficiency of the SLN dispersions was found to be in the range from 72.50 to 86.40%. The in vitro release of ramipril from the SLN dispersion was found to be in the range of 54.90 to 81.40% at the end of 12 hours. The study concluded that solid lipid nanoparticles provide controlled release of the drug and these systems are used as drug carriers for lipophilic drugs, to enhance the bioavailability of poorly water-soluble drugs through nanoparticles, as a drug delivery system (14)

Swamy *et al., (2020)* Irbesartan loaded SLNs were prepared by hot homogenization followed by the ultra sonication using Dynasan-112, Soya lecithin, Chloroform, methanol and Polaxomer 188. The result showed that the particle size was found between the ranges from 217 nm to 344 nm and PDI was from 0.163 to 0.297 nm for different formulation with different concentration. The entrapment efficiency of the SLN dispersions was found to be in the range from 72.26 to 93.68. In vitro release of

irbesartan from IS-SLNs was studied in pH 6.8 phosphate buffer by dialysis method. In pH 6.8 phosphate buffer, the cumulative % of release from formulations F1-F8 was 66.26%, 72.09%, 74.24%, 77.05%, 80.45%, 85.47%, 86.07% and 90.03%, respectively in 24 hours. The study concluded that solid lipid nanoparticles provide controlled release of the drug and these systems are used as drug carriers for lipophilic drugs, to enhance the bioavailability of poorly water-soluble drugs (15)

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CHAPTER - 3

DRUG AND POLYMER PROFILE

3. DRUG AND POLYMER PROFILE

3.1 OLMESARTAN MEDOXOMIL:

Olmesartan medoxomil is a prodrug of Olmesartan, a selective AT1 subtype angiotensin-II receptor antagonist for the treatment of hypertension. It belongs to class II under BCS.

- **Chemical name :** Olmesartan medoxomil (Wilson and Gisvold's, 1998) is described chemically as 2,3-dihydroxy-2-butenyl 4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5- ylphenyl)benzyl]imidazole5-carboxylate, cyclic 2,3-carbonate (1).
- **Molecular formula:** $C_{29}H_{30}N_6O_6$
- **Molecular weight**: 558.59
- **Structural formula :** The Olmesartan molecule includes one tetrazole group (a 5-member heterocyclic ring of four nitrogen and one carbon atom) and one imidazole group (a 5-membered planar heterocyclic aromatic ring of two nitrogen and three carbon atoms, classified as an alkaloid).

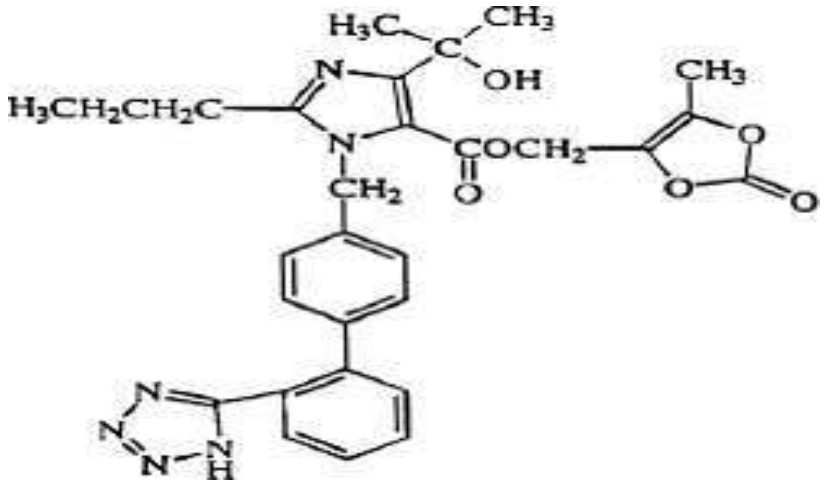

Figure 3.1 Chemical structure of Olmesartan medoxomil

- **Melting point:** 175-180 °C
- **Physical state:** White crystalline powder.
- **Solubility:** It is freely soluble in chloroform and in glacial acetic acid, slightly soluble in methanol and in acetonitrile and practically insoluble in water, ethyl acetate and in n-hexane.
- **Mechanism of action:** Olmesartan medoxomil (OLM), a prodrug, is hydrolyzed to Olmesartan during absorption from the gastrointestinal tract. It works by blocking the binding of angiotensin II to the AT1 receptors in vascular muscle; it is therefore independent of angiotensin II synthesis pathways, unlike ACE inhibitors. By blocking the binding rather than the synthesis of angiotensin II, Olmesartan inhibits the negative regulatory feedback on renin secretion. As a result of this blockage, Olmesartan reduces vasoconstriction and the secretion of aldosterone. This lowers blood pressure by producing vasodilation, and decreasing peripheral resistance.
- **Pharmacokinetics:**

➢ **General:** Olmesartan medoxomil is rapidly and completely bioactivated by ester hydrolysis to olmesartan during absorption from the gastrointestinal tract. Olmesartan is a selective AT1 subtype angiotensin II receptor antagonist. Olmesartan appears to be eliminated in a biphasic manner with a terminal elimination half-life of approximately 13 hours. The absolute bioavailability of Olmesartan is approximately 26%. Food does not affect the bioavailability of Olmesartan. After oral administration, the peak plasma concentration (C_{max}) of Olmesartan is reached after 1 to 2 hours.

➢ **Metabolism and Excretion:** Following the rapid and complete conversion of Olmesartan medoxomil to Olmesartan during absorption, there is virtually no further metabolism of Olmesartan. Total plasma clearance of Olmesartan is 1.3 L/h, with a

renal clearance of 0.6 L/h. Approximately 35% to 50% of the absorbed dose is recovered in urine while the remainder is eliminated in feces via the bile.

➢ **Distribution:** The volume of distribution of Olmesartan is approximately 17 L. Olmesartan is highly bound to plasma proteins (99%) and does not penetrate red blood cells. The protein binding is constant at plasma Olmesartan concentrations well above the range achieved with recommended doses.

- **Pharmacodynamic:** Olmesartan medoxomil doses of 2.5 to 40 mg inhibit the pressor effects of angiotensin I infusion. The duration of the inhibitory effect was related to dose, with doses of Olmesartan medoxomil > 40 mg giving > 90% inhibition at 24 hours. Plasma concentrations of angiotensin I and angiotensin II and plasma renin activity (PRA) increase after single and repeated administration of Olmesartan medoxomil to healthy subjects and hypertensive patients. Repeated administration of up to 80 mg Olmesartan medoxomil had minimal influence on aldosterone levels and no effect on serum potassium.

➢ **Dosage and administration:** Dosage must be individualized. The usual recommended starting dose of Benicar is 20 mg once daily when used as monotherapy in patients who are not volume-contracted. For patients requiring further reduction in blood pressure after 2 weeks of therapy, the dose of Benicar may be increased to 40 mg. Doses above 40 mg do not appear to have greater effect. Twice-daily dosing offers no advantage over the same total dose given once daily.

- **Adverse effects:** The incidence of adverse effects is reported as similar to placebo. Olmesartan is contraindicated in pregnancy and can cause injury and even death to the developing fetus. It causes increases in serum creatinine or blood urea nitrogen in patients with unilateral or bilateral renal artery stenosis. Rarely, Olmesartan can cause severe gastrointestinal issues. The symptoms, which include nausea, vomiting, diarrhea,

weight loss and electrolyte abnormalities are common among those who have celiac disease (2).

- **Marketed products:** Benicar, Olmetec, WinBP, Golme, Erastapex, Olsart.

3.2 POLYMER PROFILE:

- **POLOXAMER 188:** Poloxamers generally occur as white, waxy, free-flowing prilled granules, or as cast solids. They are practically odorless and tasteless.(3).

Identification, Chemical and physical properties:

Structure:

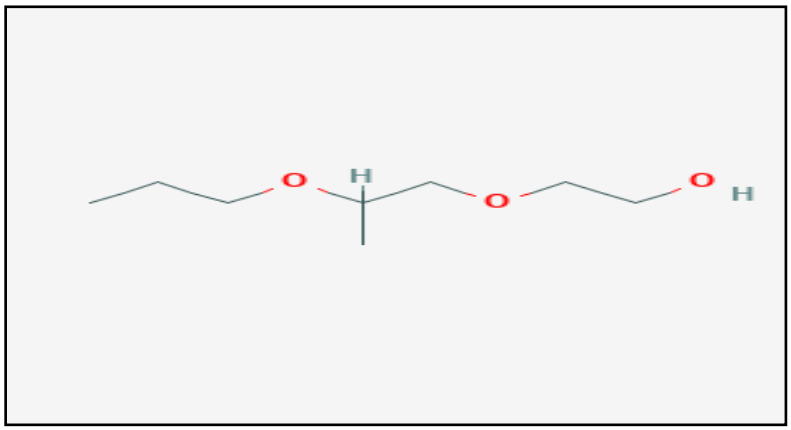

Figure 3.2 Chemical structure of Poloxamer 188

IUPAC Name: 2-(2-propoxypropoxy) ethanol

Chemical Name: a-Hydro-o-hydroxypoly(oxyethylene)poly(oxypropylene) poly-(oxyethylene)

Synonym: Lutrol; Monolan; Pluronic; poloxalkol; poloxamera

Molecular weight: 162.23 g/mol

Density: 1.06 g/cm^3 at 25 ºC

Melting point: 52-57 ºC

Solubility: Freely soluble in water and ethanol (95%)

Functional categories: Dispersing agent, emulsifying agent, solubilizing agent, tablet lubricant, wetting agent.

Applications: In pharmaceutical formulations

✓ Use as emulsifying or solubilizing agents.

✓ Use as wetting agents, in ointments, suppository bases and gels and as tablet binders and coatings.

✓ Use as an emulsifying agent for fluorocarbons,

✓ Use as artificial blood substitutes and in the preparation of solid-dispersion systems. Used in drug-delivery systems.

✓ Therapeutically, Poloxamer 188 is administered orally as a wetting agent and stool lubricant in the treatment of constipation; it is usually used in combination with a laxative such as danthron.

- **STEARIC ACID:** Stearic acid is a hard, white or faintly yellow-colored, somewhat glossy, crystalline solid or a white or yellowish white powder. It has a slight odor and taste suggesting tallow.

➢ **Synonyms:** Cetylacetic acid; Crodacid; E570; Edenor; Emersol; Hystrene; Industrene; Kortacid ; Pearl Steric; Pristerene; stereophonic acid; Tegostearic.

➢ **Chemical name:** Octadecanoic acid

➢ **Empherical formula:** $C_{18}H_{36}O_2$

➢ **Solubility:** Soluble in water, diethyl ether, acetone, ethanol, dimethyl formamide.

➢ **Functional category:** Emulsifying agent; solubilizing agent; tablet and capsule lubricant.

➢ **Applications in pharmaceutical formulations:** Stearic acid is widely used in oral and topical pharmaceutical formulations. It is mainly used in oral formulations as a tablet and capsule lubricant; although it may also be used as a binder or in combination with shellac as a tablet coating. It has also been suggested that stearic acid may be used as a sustained-release drug carrier. In topical formulations, stearic acid is used as an emulsifying and solubilizing agent. When partially neutralized with alkalis or triethanolamine, stearic acid is used in the preparation of creams. The partially neutralized stearic acid forms a creamy base when mixed with 5–15 times its own weight of aqueous liquid; the appearance and plasticity of the cream being determined by the proportion of alkali used. Stearic acid is used as the hardening agent in glycerin suppositories. Stearic acid is also widely used in cosmetics and food products (4).

- **SOYA LECITHIN:** Lecithins vary greatly in their physical form, from viscous semiliquids to powders, depending upon the free fatty acid content. They may also vary in color from brown to light yellow, depending upon whether they are bleached or unbleached or on the degree of purity. When they are exposed to air, rapid oxidation occurs, also resulting in a dark yellow or brown color. Lecithins have practically no odor. Those derived from vegetable sources have a bland or nutlike taste, similar to that of soybean oil. Soya lecithin extraction utilizes chemicals such as acetone and hexane (5)

➢ **IUPAC name:** (2-nonanoyloxy-3-octadeca-9,12-dienoyloxypropoxy)-[2-(trimethylazaniumyl)ethyl]phosphinate

➢ **Molecular Formula:** $C_{35}H_{66}NO_7P$

- **Molecular Weight:** 643.9
- **Solubility:** Soya lecithins are soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids. They are practically insoluble in cold vegetable and animal oils, polar solvents, and water. When mixed with water, however, soya lecithins hydrate to form emulsions.
- **Functional Category:** It's generally used as an emulsifier, or lubricant, when added to food, but also has uses as an antioxidant and flavor protector.

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CHAPTER - 4

AIM AND OBJECTIVES

4. AIM AND OBJECTIVES

- **Aim of the study:** Formulation and evaluation of Olmesartan loaded solid lipid nanoparticles for enhancing oral bioavailability
- **Objectives of the study:**

  The objective of the study is

  - ✓ To develop Olmesartan loaded Solid Lipid Nanoparticles by hot homogenization method followed by the ultrasonication.
  - ✓ Characterization and selection of best formulation in terms of particle size, entrapment efficiency, drug loading, *In-vitro* release study, and *In vivo* study.

4.1 PLAN OF WORK:

- Literature review on National and International Context

- **PRE FORMULATION STUDY:**

- **Organoleptic Properties:** (color, order, taste)
- **Solubility studies:** (The purpose of the test is to determine how much of a solvent can be dissolved in a solute; in other words, the highest concentration of a solute in a solvent. From a pharmaceutical perspective solubility tests can be used to determine: Maximal concentration that can be used in an in vitro activity assay)
- **Melting point Determination:** (The purpose of melting and boiling points in a lab experiment is to use them to help identify unknown substances. By taking a melting point of an unknown solid, we can compare it to a list of potential solids and their melting points and make a match to identify the solid)
- **Determination of p^H:** (This is because the formula used to calculate p^H approximates the negative of the base 10 logarithms of the molar concentration of hydrogen ions in the solution. More precisely, p^H is the negative of the base 10 logarithms of the activity of the hydrogen ion.)
- **DSC:** (This allows the detection of transitions such as melts, glass transitions, phase changes, and curing. DSC is used to determine the thermal phase transition in samples and interactions between the components of hydrogels, cyclodextrins, lipids, and surfactants)
- **FTIR Spectroscopy (Drug only):** (FTIR analysis is used to: Identify and characterize unknown materials (e.g., films, solids, powders, or liquids) Identify contamination on or in a material (e.g., particles, fibers, powders, or liquids) Identify additives after extraction from a polymer matrix)

- **FORMULATION DEVELOPMENT:**
- Preparation of Olmesartan loaded Solid Lipid Nanoparticles by hot homogenization method followed by the ultrasonication.
- ***IN-VITRO*** **EVALUATION AND OPTIMIZATION:**
- **Particle size analysis:** (Particle size analysis gives information on the size distribution of particles. This can be used to calculate different properties of a particle and how they will act under certain conditions.)
- **Drug loading:** (Drug-loading content and drug-loading efficiency are two important parameters of nanomedicines. Drug-loading content reflect the mass ratio of drugs to nanomedicines, and drug-loading efficiency reflects the utilization of drugs in feed during the nanomedicine-preparation process)
- ***In-vitro*** **Drug release studies:** (*In-vitro* drug release testing, a measure of the release of the active pharmaceutical ingredient (API) from the drug product matrix in a controlled laboratory environment.)
- **Percentage yield:** (The calculation of the percent yield in chemical reactions is very important because it can help us determine and be conscious of, and possibly correct, the conditions that made the actual yield and theoretical yield differ.)
- **Zeta potential:** (Zeta potential measurements to improve formulation stability and shelf life and reduce formulation time and cost.)
- **FTIR Spectroscopy (Drug + excipients):** (FTIR analysis is used to: Identify and characterize unknown materials.)
- Drug entrapment efficiency
- **IN VIVO RELEASE STUDY**
- Pharmacokinetic study

CHAPTER - 5

METHODOLOGY

5. METHODOLOGY

5.1 MATERIALS USED:

All the drugs and chemicals used are of analytical grade and the manufacturers for respective chemicals are listed below.

Name of the Chemicals: Olmesartan, Stearic Acid, Soya Lecithin, Chloroform, Methanol, Poloxamer 188

5.2 INSTRUMENTS USED:

All the instruments used in the practical works conducted throughout the project and the respective manufacturers are enlisted below.

Table 5.1: List of instrument and their manufacturers

Instruments	Company name
Digital weighing balance	Denver Instrument, USA
Magnetic Stirrer	Rolex, India
Homogenizer	IKA T25 Digital Ultra Turrax, Germany
Refrigerator	Godrej, India
Magnetic stirrer with hot plate	Rolex; India
UV Visible Spectrophotometer	Shimadju, Model No: UV 1800 240V, Japan
FT-IR	Alpha, Bruker Model No: 10059736, Germany
Melting point apparatus	Macroscientific works 10A/UA, Janwahar Nagar, Delhi-11007

5.3 PREFORMULATION STUDY OF THE DRUG:

- **Organoleptic Properties:** The organoleptic properties color, odor, taste were evaluated.
- **Fourier Transform Infrared Spectroscopy (FTIR):** The FT-IR analysis of the pure drug, excipients, drug-excipients mixture were carried out with the FT-IR instrument.
- **Solubility of Olmesartan medoxomil:** A minute quantity of the drug was taken on a test tube and the solubility of the drug was determined by dissolving the drug in various solvents like water, acetone, methanol, ethanol, chloroform, ethyl acetate, glacial acetic acid, etc.
- **Melting point:** A little amount of the drug sample in a dry capillary tube of 1 mm internal diameter forming a column about 3mm high. Heat the melting point apparatus to a temperature (5-10) °C below the expected temperature of melting and adjust the heating so that the temperature in the chamber rises about 10 °C per minute. (1)
- **Differential Scanning Caloriemetry(DSC):** DSC will be used to determine the nature and specification of the crystallinity of drugs and excipients through the measurement of glass transition temperature and melting point temperature and their associated enthalpies. This technique will be used to study the physical and chemical interaction between drug and excipients. The required amount of drug, drug- polymer mixtures will be taken and then the samples will be sealed in aluminium pans analyzed in an atmosphere of airflow rate 25 ml/min. A temperature range of 30 °C to 4000 °C will be used where the rate of heating is 10 °C/min. (2)
- **Determination of λ_{max}:** 100 mg of Olmesartan medoxomil was accurately weighed and dissolved in 100 ml of methanol in volumetric flask. 10 ml of above solution was diluted with 100 ml of methanol (=100 µg/ml) in separate volumetric flask and scanned

for maximum absorbance in UV double beam spectrophotometer in the range from 200 to 400 nm, using methanol as blank. The λ_{max} of the drug was found to be 257 nm. (3)

- **Standard Curve for Olmesartan medoxomil:** 100 mg of Olmesartan medoxomil was accurately weighed and dissolved in 50 ml methanol. The solution was sonicated for 10 min and final volume was adjusted to 100 ml to give stock solution-I (1000 µg/ml concentration). 10 ml of stock solution-I was placed in 100 ml volumetric flask and volume was adjusted with methanol to give stock solution-II of 100µg/ml concentration. Stock solution-II was further diluted with methanol to get working standard solution of 4, 6, 8, 10, 12, µg/ml of Olmesartan medoxomil to construct Beer's law plot for the pure drug. The absorbance of the solutions was measured at 257 nm using UV-visible spectrophotometer. A graph of concentration v/s absorbance was plotted.

5.4 PREPARATION OF OLMESARTAN LOADED SOLID LIPID NANOPARTICLES:

OM-loaded SLNs were prepared by hot homogenization method followed by the ultrasonication. OM, stearic acid, and soya lecithin were dissolved in 10 ml mixture of chloroform and methanol (1:1). Organic solvents were completely removed using a rota evaporator. The drug embedded lipid layer was molten by heating at 5 °C above melting point of the lipid. An aqueous phase was prepared by dissolving Poloxamer 188 in double distilled water and heated to same temperature (based on lipid melting point) of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out at required rpm using homogenizer for required time. The coarse hot oil in water emulsion so obtained was ultra-sonicated using a 12 T probe sonicator for 20 min. The hot formulation was allowed to cool to room temperature to obtain olmesartan medoxomil loaded solid lipid nanoparticles formulation. (4)

Table 5.2: Composition of Olmesartan medoxomil-loaded SLNs

Ingredients (mg)	Formulation code for SLNs				
	F1	F2	F3	F4	F5
Olmesartan	10	10	10	10	10
Stearic acid	50	100	150	200	250
Soya lecithin	100	100	100	100	100
Chloroform:Methanol (1:1) (ml)	10	10	10	10	10
Poloxamer 188	150	150	150	150	150
Distilled water	10	10	10	10	10

5.5 CHARACTERIZATION AND OPTIMIZATION OF SOLID LIPID NANOPARTICLES FORMULATIONS:

- **Measurement of particle size and polydispersity index:** The particle size analysis of the solid lipid nanoparticles and polydispersity index (PDI) was determined by dynamic light scattering using Zetasizer (Malvern Nano S90). The formulations was diluted with distilled water before measurement.

- **Zeta potential:** Zeta potential, which reflects the surface electric charges of the particles, will be determined using zeta potential measurement (Malvern), which applies the principle of electrophoresis. An electric field will be applied across the dispersed particles in which the particles will migrate to the oppositely charged electrode with a velocity that is proportional to the value of the zeta potential. The velocity of migration will be measured using the technique of Laser Doppler Anemometer. The magnitude of zeta potential reflects the degree of electrostatic repulsion between charged particles. For the dispersion system, a high degree of zeta potential, negative or positive indicates resistance of the particles to aggregation, and this denotes a stable system. (5)

- **Drug loading (DL)**: A 2 ml of each optimized formulation will be diluted to 10 ml using ethanol. The absorbance of the drug in all dilutions will be measured at 257 nm. The drug content in each formulation will be obtained from calibration curve.

Determinations will be done in triplicate. (4)

- **Drug entrapment efficiency (EE):** The entrapment efficiency of the optimized formulations will be done in triplicate by obtaining the concentration of free drug in the aqueous phase of solid lipid nanoparticles formulation following centrifugation of 2 ml of each formulation. Centrifuging of the formulation will be done at high speed (15000 rpm) for 30 min at room temperature. Absorbance of the aqueous solution will be taken at a wavelength of 257 nm. The percent entrapment efficiency will be calculated as follows:

$$\% \text{ EE} = \frac{\text{Total drug content} - \text{free drug content}}{\text{Total drug content}} \times 100$$

- ***In-vitro*** **drug release study:** The dialysis membrane diffusion technique will be used. The required amount of the nanosuspensions will be placed in the dialysis membrane (Mw cutoff 12,000–14,000 Hi- media), fixed in a Kiserychien apparatus of surface area and receptor volume of 20 ml. The entire system will be kept at 37 ºC with continuous magnetic stirring. Samples will be withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. The amount of drug dissolved will be determined with UV spectrophotometry. (6)
- ***In Vivo*** **release study:**
- **Pharmacokinetic Studies on Optimized Solid Lipid Nanoparticles Formulations:** A single-dose bioavailability study will be designed in male Sprague Dawley Rats under fasting conditions. The oral bioavailability of the optimized OM-SLN formulation will be estimated in male Sprague Dawley rats with an oral dose of 10 mg/ kg body weight. All experimental procedures were reviewed and approved by the institutional animal ethical committee. Male Sprague Dawley rats weighing 200–250 g were proposed for study (06 animals per group). Blood samples will be withdrawn by

retro-orbital venous plexus puncture at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h post-dose. About 0.5 ml of blood samples will be withdrawn in Eppendorf tubes and centrifuge at 3000 rpm for 30 min. The serum samples will be separated and stored at –20 °C until analysis by HPLC. (7)

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CHAPTER - 6

RESULTS AND DISCUSSIONS

6. RESULTS AND DISCUSSIONS

6.1 PREFORMULATION STUDY OF DRUG:

- **Organoleptic properties:** The organoleptic properties like colour, odour, and taste were observed and the results were compared with the official requirement and found to be acceptable. The results are shown in Table 6.1.

Table 6.1: Organoleptic properties of the drug

Sample	Taste	Colour	Odour	Physical State
Olmesartan	Bitter	White	Odourless	Crystalline powder

- **Solubility study:** A minute quantity of the drug was taken on a test tube and the solubility of the drug was determined by dissolving the drug in various solvents like water, acetone, methanol, ethanol, chloroform, ethyl acetate, glacial acetic acid, etc. The results are shown in Table 6.2

Table 6.2: Solubility of the drug

Solvent	Solubility
Water	Insoluble
Methanol	Slightly soluble
Ethanol	Soluble
Chloroform	Soluble
Ethyl acetate	Insoluble
Glacial acetic acid	Soluble

- **Melting point determination:** The melting point was determined by using the melting point apparatus with the help of a capillary tube. The melting point of Olmesartan was found to be 178 ºC. The melting range was compared with the official requirement and is found to be acceptable.

6.2 DETERMINATION OF λ_{MAX} OF OLMESARTAN MEDOXOMIL:

The λ_{max} of the Olmesartan medoxomil was found to be 257 nm in methanol.

6.3 CALIBRATION CURVE OF OLMESARTAN MEDOXOMIL:

The absorbance of Olmesartan medoxomil was measured in a UV spectrophotometer at 257 nm against methanol. The absorbance so obtained was tabulated (table no.) and graph was obtained by plotting absorbance v/s concentration shown in figure no 6.1.

Table 6.3: Spectrophotometric data of OLM in methanol

Concentration(µg/ml)	Absorbance(nm)
0	0
2	0.063
4	0.152
6	0.211
8	0.298
10	0.337

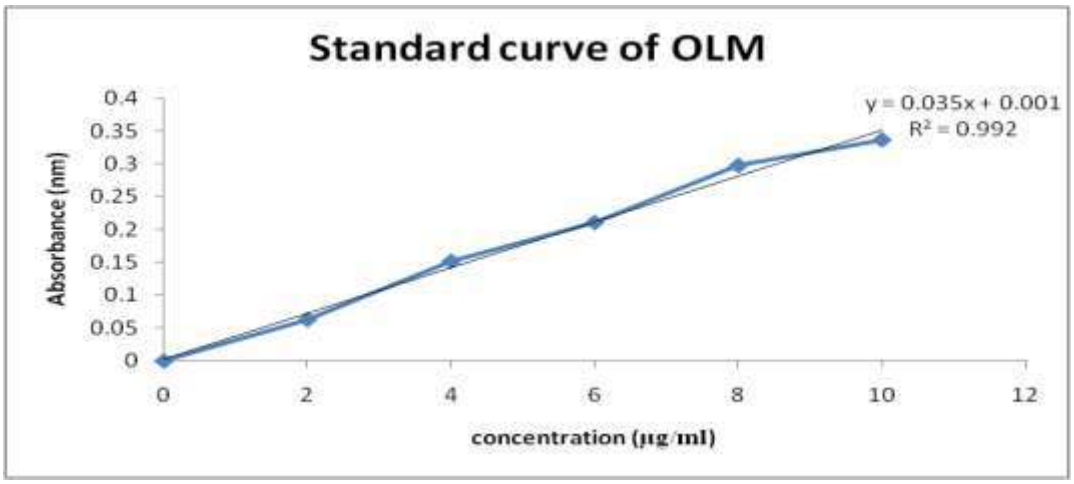

Fig 6.1: Standard curve of OLM in methanol.

6.4 FT-IR STUDY OF DRUG AND EXCIPIENT:

The physicochemical compatibility study was carried out by infrared spectroscopy. IR spectral analysis of Olmesartan showed peaks at 2963.74 cm-1 (C-H Stretching, aromatic), 763.34 cm-1 (C-H bending, aromatic), 1735.62 cm-1 (C=O stretching, ester), 1705.62(N-H Bending), 1051.50(O-H Bending, alcohols), 1705.62(C=C stretching, aromatic) confirmed the purity of the drug with standard. After the interpretation drug and drug-polymer mixture was found to be compatible as shown in the figure (table).

Table 6.4: Interpretation of FT-IR spectrum

Ingredients	Functional groups with wave number (cm^{-1})					
	C-H Stretching (aromatic)	C-H Bending (aromatic)	C=O Stretching (Ester)	N-H Bending	O-H Bending (alcohols)	C=C Stretching (aromatic)
Olmesartan Pure drug	2963.74	763.34	1735.62	1705.62	1051.50	1705.62
Drug and Polymer mixture	2923.69	762.86	1737.82	1705.48	1051.80	1705.48

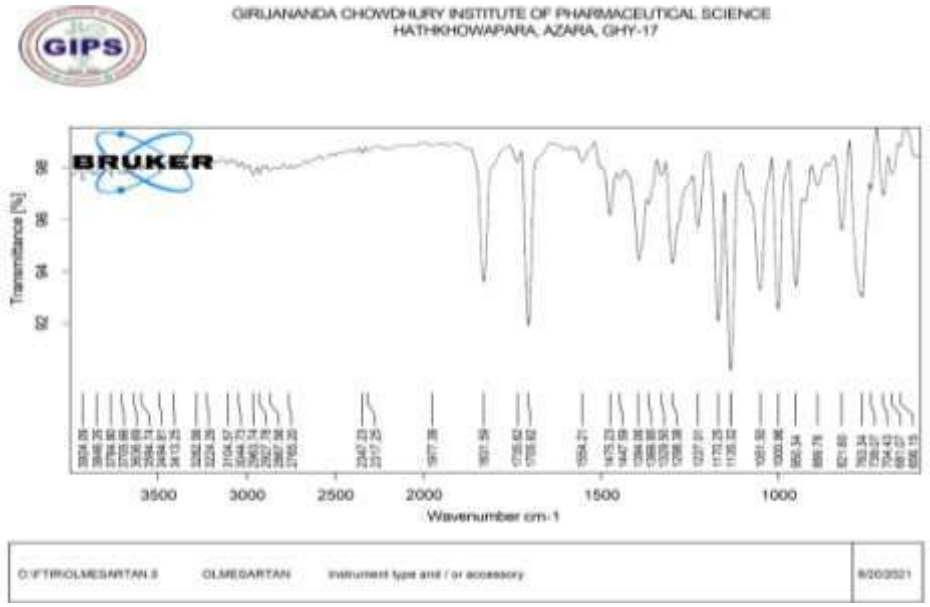

Figure 6.2: FT-IR spectra of Olmesartan

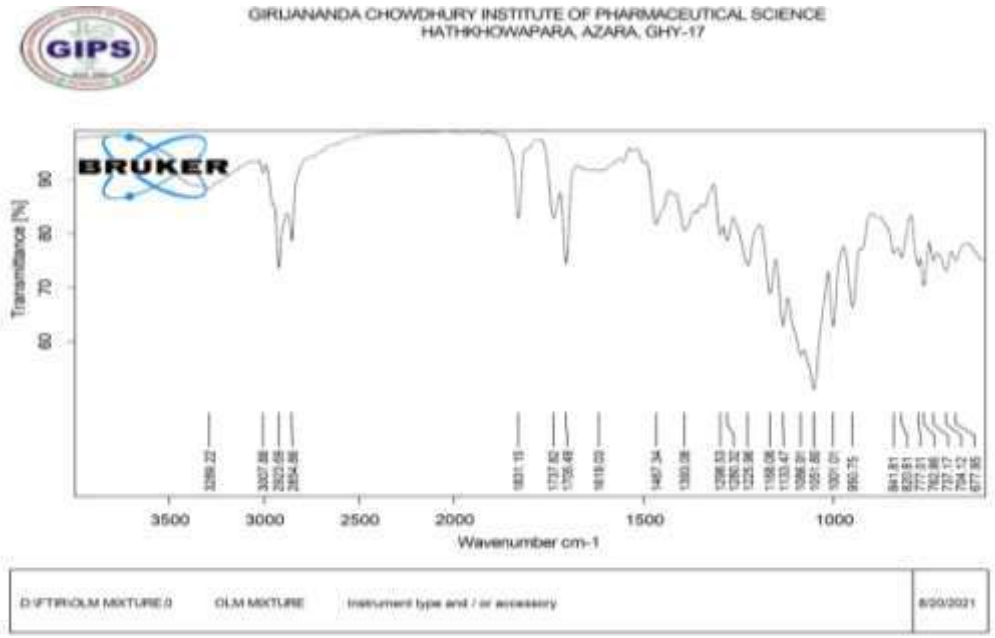

Figure 6.3: FT-IR spectra of drug and polymer mixture

6.5 CHARACTERIZATION OF FORMULATED SLNS:

All the prepared blank SLN formulations were analyzed for their particle size and PDI. During the experimentation it was seen that the formulation with rpm 12000 for 30 min was burned. Previous report had shown that particle sizes less than 300 nm are advisable for the intestinal transport (1). It has been reported (2) that a polydispersity

index value less than 0.3 is often accepted as optimum value. So from these values we can report that particle size for the rpm 12000 in 4 min is better than others.

Table 6.5: Composition of Slid lipid nanoparticles formulations

Ingredients	Amount
Stearic acid	100 mg
Soya lecithin	100 mg
Chloroform:Methanol (1:1) (ml)	10 ml
Poloxamer 188	150 mg
Distilled water	10 ml

Table 6.6: Particle size, PDI values for 30 min Homogenization

RPM	Time (min)	Particle size (nm)	PDI
5000	30	458.8	0.380
800	30	443.2	0.354
12000	30	377.7	0.413

Table 6.7: Particle size, PDI values for 4 min Homogenization

RPM	Time (min)	Particle size (nm)	PDI
5000	4	379.2	0.336
800	4	344.9	0.598
12000	4	203.5	0.228

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CHAPTER - 7

CONCLUSION

7. CONCLUSION

Nanoparticulate drug delivery system is a promising approach for the formulation of the Olmesartan loaded solid lipid nanoparticles. The drug Olmesartan medoxomil is highly lipophilic (log P 5.5) and a poorly water-soluble drug with absolute bioavailability of 26%. High lipophilicity (log P>5) often contributes to low solubility, and poor oral absorption. One of the reasons for poor bioavailability is poor solubility of drugs as drug should be present in solution form at absorption site. SLN are defined as aqueous colloidal dispersion of solid lipids, stabilized in an aqueous dispersion by surfactants or polymers. The use of biocompatible lipids, ability to encapsulate lipophilic molecules and their small size due to which they can enter the lymphatic system and thus bypass the first pass effect makes them an attractive delivery systems for the poorly aqueous soluble drugs. Lipophilic drugs enter the lymphatic systems in combination with the triglyceride core of the chylomicrons. The lipid vesicles enhance the lymphatic transport of the incorporated actives by increasing lymph formation, promoting lymph flow rate and stimulating the production of chylomicrons. Transport of drugs directly into systemic circulation through the intestinal lymphatics avoids presystemic metabolism and therefore enhances the bioavailability. In this project work SLNs were prepared by hot homogenization method by using stearic acid, soya lecithin, chloroform, methanol, and poloxamer 188.

Olmesartan which was supplied for the study is a standard drug which was evaluated by IR- spectroscopy as well as drug melting point. The melting point of the drug was found to be 178ºC which is more close to the actual melting point of the drug. The solubility study revealed that Olmesartan is very soluble in chloroform, ethanol and in glacial acetic acid, slightly soluble in methanol and insoluble in water, ethyl acetate.

From the obtained result it is concluded that the blank formulation with rpm 12000 for 4 min was better formulation with particle size 203.5 nm and PDI 0.228. Hence this rpm with that specific time will be suitable parameters to suggest in carrying out the formulations of Olmesartan loaded solid lipid nanoparticles.

- # LIST OF PUBLICATIONS

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- **PUBLICATIONS ON PROCESS:**

1. Rahman S.S., **Baishya B.**, Sarma A.: Selection of different excipients for controlled release formulations of Pantoprazole through drug–excipient compatibility testing: Research Journal of Pharmacy and Technology. (Under review process)
2. Rahman S.S., **Baishya B.**, Medhi S.: Review on microemulsion used as a novel drug delivery system: International Journal of Pharmaceutical Sciences and Research. (Under Review process).