CHAPTER 1 - INTRODUCTION

In the present situation, conventional medication is investigating a novel drug delivery system due to the defective factors like less solubility, reduced absorption, rapid first pass metabolism, more fluctuation plasma drug concentration and variation in concentration of drug in plasma and also due to food – drug interaction, patient related factors like improper prescription, no patient compliance, more toxicity, which playing a vital role in loss of desirable in-vivo pharmacological effect. These consequences lead to failed conventional delivery system which paved way for novel drug delivery system like Nanostructured Lipid carrier (NLC) [1].

The oral medication defects leads to use of lipid as a delivery or carrier system for less water soluble, lipophilic drugs (BCS Class II drugs like Carvedilol and Simvastatin), which leads to potential increase in solubility as well as bioavailability of above class drug [2].

In this era, lipid nanoparticles like NLC are the potential carrier than existing lipid particulate systems like Liposome and Polymeric Nanoparticles. NLC ensures good stability both physically and chemically, excellent drug loading capacity, good release controlling carriers and it can use in various administrative routes like oral, ocular, parenteral, dermal, rectal and pulmonary [3-5].

NLCs shows the following unique properties such as

- Good physiochemical diversity compared to all other lipid carrier system
- Good biocompatibility when compared to Solid Lipid Nanoparticles (SLN)
- High potential ability to increase the bioavailability of poorly aqueous soluble drugs,
- Good target ability by selective lymphatic uptake mechanism
- Feasibility of scaling-up to large scale [6].

1.1. Nano structured lipid carrier

Nanostructured lipid carriers (NLCs) are the novel drug carrier and these are the generation next to solid lipid nanoparticles (SLNs). NLC carrier contains two mixtures of solid lipid connected with the spatially arranged liquid lipid phase. Due to this spatial alternative arrangement of solid lipid and liquid lipid the drug loading
efficiency has enhanced, and also drug load will overcome the crystallinity character of lipid matrix which has high ability to convert solid lipid to crystals.

In NLC the particle will be in amorphous form due to presence of both solid and liquid lipid in the form of chain or in blocks, which prevents the expulsion of drug in recrystallization state during storage. NLC acts as a good alternative carrier for conventional systems such as solutions, suspensions and ointments [7, 8].

NLC is a micellar colloidal drug carrier nanoparticle dispersions consist of particles in the range of 10–500nm in diameter. To obtain a good NLC matrix the solid lipids and liquid lipids are mixed together. Due to presence of oil in NLC there will be a melting point depression will be there when compared to SLN. Solid lipid and liquid lipid ratio in NLC can be extended upto 95%.

NLC are not spherical in shape as other lipid carrier. They have liquid lipid droplets embedded in solid lipid particles which bind together with the help of surfactant. Usually they are seen in blocks or in chain form as shown in Figure 1.1[11].

Figure 1.1: Difference between the Structure of SLN and NLC
Advantages of NLC

- Excellent loading efficiency, good permeability and high stability.
- It protects the sensitive drug molecules from degradation like stomach acid degradation, enzymatic degradation.
- Due to slower degradation rate in in-vivo condition it provides desires and better controlled drug release.
- It provides superior protection to the loaded or encapsulated drugs [12].
- It shows excellent tolerability and acceptability due to its structural changes by solid lipid and liquid lipid modification.
- For its highly lipophilic in nature NLCs has been used to administer lipophilic drugs [13].
- It has more drug loading capacity, due to its structural mixture of solid and liquid lipids. So that they can’t fit together very well as crystal form, (i.e.) it has more flaws in its molecular structure.
- Compared to polymeric and other nanoparticle, NLC has lower toxicity because of the absence of the solvents in the production process
- It use very low cost of excipients so that NLC formulation is cost effective and can ease to scale up the batch to manufacturing scale.
- It shows excellent tolerability in different biological environment condition like change in pH and temperature condition.

Disadvantage of NLC

- Phase separation of lipid and aqueous phase takes place due to precipitation of tiny oily nanocompartments in the liquid lipid phase.
- Risk of gelation, particle growth due to improper storage condition [14-15].

A Reverse Micellar method to form nanocarrier is subjected for the encapsulation of hydrophobic drugs in lipid nanoparticle, and to enhance its solubility. Surfactants in non-polar medium forms Reverse Micelles (RM) which acts as multimolecular entities to form nanoparticle.

NLCs have high ability to encapsulate hydrophobic molecules in an oily core of lipid nano droplets. Both hydrophobic and hydrophilic drugs can be encapsulated to
form a stable lipid core. Therapeutic action of the drug molecule is effected by both the drugs and lipid carrier system.

Lipid carriers are novel drug delivery system with high therapeutic efficacy, good stability, minimum toxicity and side effects with good patient compliance. Lipid carrier colloidal systems, due to their high stability, were used for drug targeting by lymphatic uptake mechanism and also for topical application.

NLC shows small lipid platelet structure due to more oil content between solid lipids. However, both SLN and NLC possess numerous features which are advantageous for topical route of application [16-18]. Different types of NLC are shown in Figure 1.2.

![Figure 1.2: Different types of NLC](image)

1. Imperfect type
2. Amorphous type
3. Multiple types

1.2. Nanostructured Lipid Carriers Classification [11, 18]

NLC - Imperfect type

Imperfect type of NLC was produced by blending both liquid lipid and solid lipid to enhance the capacity of drug loading efficiency. On fabrication of NLC, the liquid lipids nanoparticles were cooled to produce a crystalline molten state at room temperature condition.
NLC - Multiple type

Multiple type of NLC was produced by using more quantity of oil in solid lipid. It will enhance the solubilization of certain poorly soluble drugs. If drug is not soluble in lipid it will results in precipitate formation.

NLC - Amorphous type

Amorphous type NLCs were formulated to reduce the expulsion of drug by blending different variety of lipids like isopropyl myristate and Hydroxyloctacosanyl hydroxy stearate. The lipid matrix will be in amorphous state.

1.3. Materials used in Nanostructured lipid carriers

The materials used for preparation of NLCs are solid lipid, liquid lipid and surfactants. Initially they have to meet the compatibility between the mixtures for a good formulation. The solid lipid used in NLCs should contain melting point more than 40ºC. It should be biodegradable in nature and must have the stability on body temperature condition [11]. Some of the materials used in NLC formulation are tabulated in Table 1.1.

<table>
<thead>
<tr>
<th>Solid lipid</th>
<th>Liquid Lipid</th>
<th>Emulsifier</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lipophilic</td>
</tr>
<tr>
<td>Bees Wax</td>
<td>Cetiol V</td>
<td>Span</td>
</tr>
<tr>
<td>Compritol</td>
<td>Softigen</td>
<td>20,40,60</td>
</tr>
<tr>
<td>Witepsol</td>
<td>Oleic acid</td>
<td>Tween 80</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Miglyol 181</td>
<td>60</td>
</tr>
<tr>
<td>Carnauba Wax</td>
<td>Captex 225</td>
<td></td>
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<tr>
<td>Inwitor</td>
<td>Isopropyl myristate</td>
<td></td>
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<tr>
<td>Dynasan</td>
<td>Liquid Paraffin</td>
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</table>

1.4. Mechanism of drug release in NLC

The medication or drug entrapped in NLC bypasses the hepatic metabolism and target the desired site of action. The drug encapsulated lipid molecule achieves the specific site of action by binding with the predetermined receptors and enters the channel reasonable for its activity. NLC get through the external layer of cell
membrane and they were consumed by the biological fluids of the cell. Due to interaction of biological fluids the lipid layer become feeble and nourishes the drug release in specific site of action in a controlled manner. The mechanism of NLC drug release is shown in Figure 1.3 [19].

![Figure 1.3: Mechanism of drug release from NLC](image)

1.5. Method of preparation of NLC [1, 10, 11, 19, 20]:

1.5.1. Hot homogenization method

This method is carried out by melting the lipid above its melting point. Then weighed quantity of drug is dispersed in the above melt. A pre-emulsion is formed. An aqueous phase containing surfactant was heated at same temperature and kept under homogenization by using high speed homogenizer. To this aqueous phase add lipid phase with high homogenization. Temperature above the melting point of lipid was maintained throughout the process. There will be a reduction in particle size due to the maintenance of constant temperature and homogenization speed. The main disadvantage is when there is an increase in high temperature there is degradation in drug characteristics. After completion of homogenization process the formulation is cooled to room temperature, which leads to solidification of the particles.
1.5.2. Cold homogenization method

The drug is dispersed in a melted lipid and then it is cooled to room temperature, and then the cooled solid lipid ground to microparticles. Then the cold surfactant solution is prepared. To this cold surfactant solution the lipid microparticle solution is dispersed to get a presuspension. Then the presuspension is kept under homogenization at room temperature. The homogenization force and gravitation force exhibited in particles helps to break the lipid microparticles into NLC. In this process drug degradation will not occur when compared to hot homogenization technique.

1.5.3. Solvent evaporation technique

A weighed quantity of lipid is dissolved in an organic solvent like ethanol, methanol and cyclohexane. Prepared organic solvent is then emulsified with aqueous phase containing emulsifier or surfactant. The Final solution is kept in high-pressure homogenization for 10-15 min. Organic solvent is then removed from the emulsion mixture by evaporating the mixture under reduced pressure of about 40–60 mbar. NLC dispersion is formed after evaporation of organic solvent. NLC carrier is formed due to the precipitation of the lipid in the aqueous medium. The particle size diameter of about 25 nm can be achieved by this method.

1.5.4. Solvent emulsification-diffusion method

In this technique, the weighed quantity of lipid material is dispersed directly in organic phase. Emulsification process is done by dispersing the lipid material in aqueous phase containing surfactant, under high pressure by using homogenizer. Precipitation of the lipid in aqueous media is then obtained by the formation of Nanoparticles dispersion. By this technique 30-100 nm particle size diameter can be obtained.

1.5.5. Microemulsion based method

This method is based on micro emulsions dilution process. It is made by continuous stirring of optically transparent mixture at 65-70°C, which is normally made out of a fatty acid with low melting point containing surfactant, co-surfactant and water. The above hot microemulsion is dispersed into ice water under continuous mixing by magnetic stirrer. The proportion of hot microemulsion and cold water should be in the range of 1:50 and 1:25.
1.5.6. Double emulsion method

In this process, lipids get melted and the hydrophilic drugs that already dissolved in aqueous solution containing emulsifier or stabilizer is dispersed to get a primary emulsion. In the next step this primary emulsion is again dispersed into the water containing hydrophilic emulsifier under continuous stirring to get a double emulsion. The surfaces of lipid Nanoparticles are coated with lipid-PEG derivative in order to get a stabilized nanoemulsion. A major disadvantage of this technique is the formation of large number of microparticles.

1.5.7. Precipitation method

In this method, glycerides are dissolved in an organic solvent like chloroform and the solution is emulsified in an aqueous phase. After evaporation of the organic solvents, the lipid gets precipitated to form NLC.

1.5.8. High speed homogenization followed by ultra sonicatation method

Required quantities of drug, phospholipids are dissolved in suitable solvent like acetone and methanol solution containing desired quantity of fatty acids at 70°C under homogenization of 15000 rpm for 10 min to get a primary emulsion. This primary emulsion is further ultrasonicated for 15 minutes to get an O/w type of emulsion and also ultrasonication is done to avoid the crystallization of lipids. The finally obtained primary emulsion is then subsequently cooled down to room temperature with continuous stirring. The obtained NLC dispersions is lyophilized to get an amorphous NLC powders and stored in a desicator for further studies.
<table>
<thead>
<tr>
<th>SL. No</th>
<th>Methods for preparation of NLC</th>
<th>Importance</th>
</tr>
</thead>
</table>
| 1      | Hot Homogenization Method     | No use of organic solvents and feasibility to large scale production.  
     |                                 | The drug can be degraded due to application of high temperature.  
     |                                 | Due to elevated kinetic energy of the particles at homogenization speed it will leads to enhancement of particle size. Homogenous particle size |
| 2      | Cold Homogenization Method    | Highly thermolabile substance can be encapsulated.  
     |                                 | Drug degradation due to high temperature, distribution of drug into the aqueous phase during hot homogenization are overcome by this method |
| 3      | Microemulsion technique       | The micro droplets already exist in the microemulsion, so no need of mechanical energy is required to achieve nanoparticles  
     |                                 | Cost effective technique |
| 4      | Solvent evaporation method    | 25 nm diameter of NLCs can obtained by this technique  
     |                                 | No sophisticated equipment needed |
| 5      | Solvent emulsification-diffusion method | 30 - 100 nm diameter of NLCs can obtained by this technique  
     |                                 | Suitable for thermolabile drugs |
| 6      | Double emulsion method        | This technique is used mainly for hydrophilic drugs  
     |                                 | Main disadvantage is formation of more microparticle |
| 7      | Precipitation technique       | Simplified and reproducible method for formulation of NLC |
| 8      | High-speed homogenization followed by ultrasonication method | It is a low temperature solidification method, it leads to good feasibility and cost effect NLC formulation |
1.6. Mechanism of NLC in enhancing bioavailability: The behavior of NLCs in body to enhance bioavailability is discussed in the following Table 1.3

**Table 1.3: Mechanism of NLC in enhancing bioavailability [3].**

<table>
<thead>
<tr>
<th>Mechanism of NLC</th>
<th>Function of NLC in enhancing bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct uptake</td>
<td>In GIT NLC is transport by intestinal lymphatic system. NLC has long chain triglycerides which stimulate the production of chylomicron, which absorbed efficiently through transcellular route. NLC are highly lipophilic compounds transported by intestinal lymphatic system that avoids first pass hepatic metabolism, which leads to enhancement of bioavailability.</td>
</tr>
<tr>
<td>Mucoadhesion</td>
<td>NLC adhere to the mucus membrane, so that there will be increasing in residence time of carrier in mucus leads to controlled release of drug, which further leads to enhancement of bioavailability</td>
</tr>
<tr>
<td>Mixed micelle formation</td>
<td>NLC lipid is similar to dietary lipids, which induces bile secretion. This lipid on enzyme degradation will form byproduct, which blended with bile to form a mixed micelle. This mixed micelle will enhance the solubility and permeation of drugs across the membrane, which leads to increase in bioavailability.</td>
</tr>
<tr>
<td>Increased permeability</td>
<td>By adding surfactant like Poloxamer there will be increase in permeability lead to enhancement of bioavailability.</td>
</tr>
<tr>
<td>Inhibits drug degradation</td>
<td>NLC inhibits drug degradation by protecting the drug from enzymatic and chemical degradation, which leads to increase in bioavailability.</td>
</tr>
<tr>
<td>NLC through skin</td>
<td>It bypasses the first pass metabolism and more permeation will leads to enhancement of bioavailability of drugs.</td>
</tr>
</tbody>
</table>
1.7 Application of Nanostructured Lipid Carriers

1. It is, applied as topical formulation, as sustained oral dosage forms preparation, Inhalation type of drug delivery system, parenteral formulation, drug targeting, and potential way to treat cancer.
2. Treatments for skin diseases are done such as skin dermatitis, psoriasis and inflammations.
3. The carrier with high biocompatibility is used in cosmetic industries, pharmaceutical industries and biochemical industries.
4. Recently proteins, peptides, all types of hydrophilic and lipophilic drugs can be entrapped within this NLC carrier [11, 20].

1.8. NLC in transdermal drug delivery system (TDDS)

Transdermal drug delivery system is a potential non-invasive route for drug administration to maintain a uniform drug level plasma by controlling the drug delivery system, bypasses hepatic circulation, decrease frequency of dose so better patient compliance [21].

TDDS is a suitable dosage form to enhance the bioavailability of less permeable and soluble drugs. Due to the presence of skin esterase transdermal route will change the drug to their active metabolite by de-esterification mechanism. TDDS also shows very low side effect.

The advantages of TDDS are pain free dosage form, exclusion of first pass hepatic metabolism, controlling drug release for an extended period of time, self administration, and any time termination of medication is possible by simple withdrawal of dosage form from skin, in case of discontinuation of medication, enough doses or in side effects occasion.

Transdermal patch is a multilaminated adhesive system. It is a self enclosed, discrete dosage form that is topically administered dosage form with an exact dose which releases the drug in a controlled and predetermined rate into the blood plasma [22]. Stratum corneum layer acts as a barrier for the permeation of drugs through skin. Due to this only a few amount of drug will reach the systemic circulation.

Particulate carrier system like lipid nanoparticle loaded transdermal drug delivery system can overcome the barriers in permeation of drug through skin and also enhance the permeation and bioavailability of drug. Because lipid carrier and lipids
present in skin exchange the lipid in the skin surface and break the barrier in stratum corneum, which leads to enhance the permeation of drug through skin. NLCs will attach automatically to the skin surface and leached through the pores, lipid layer and reach the systemic circulation. It will act as a potential carrier system for low soluble and low permeable drug through skin [23].

Topical dosage form like ointments, creams and gels shows low predictable, high fluctuation of drug concentration in plasma, which leads to toxicity and low patient compliance. NLC assists the drug loaded lipid in the upper layer of the skin to maintain an occlusive character. This occlusive effect of NLC will form a thin film on the surface of the skin that reduces the transepidermal water loss and controlling the drug release for a prolonged period of time [24].

1.9. Mechanism of drug release from NLC loaded transdermal drug delivery system

The following rate controlling steps plays a vital role in controlling the drug release from transdermal drug delivery system

- Rate of drug diffusion from the device
- Rate of drug permeation through the stratum corneum

In NLC loaded transdermal system, appropriate mixing of solubilizers with solid lipid offers extraordinary chance to stack low soluble drugs with enhanced bioavailability and good stability. It permits controlled and predetermined release of drug from the dosage form with considerable enhancement of skin permeation, increased bioavailability when observed through in-vivo dermatopharmacokinetics.

1.10. Permeation enhancement mechanism and selection of permeation enhancers

Permeation enhancers follow the following mechanisms:

- Interruption of ordered lipids in subcutaneous layer,
- Reaction with the epidermal keratin and
- Enhancement in partitioning of the medicine into the skin.

Activities on the intercellular keratin may bring out the denaturation of the protein. This process is called as irreversible biological process [25].
Recently, the lipid nano carriers like transfersome have been accounted for the enhancement of solubility and bioavailability through the transdermal delivery. NLC stacked transdermal patches is a type of transfersomes, which has the capability to overcome the penetration barriers by squeezing themselves through the intercellular lipid on the stratum corneum [26].

NLC have a moisturizing outcome on the skin during occlusion which in turn provides good skin hydration and also good solubility and bioavailability of drug. NLC with permeation enhancers was used as a vehicle for transdermal drug delivery system [27, 28].

The occlusion factor in NLC is indirectly proportional to its particle size. It shows increase in occlusive factor with the decrease in mean particle size, i.e., nanosized lipid particles will make sure about the close contact of particles to the stratum corneum, which may enhance the penetrating power of NLC through the stratum corneum into the skin. Owing to its solid lipid and liquid lipid matrix structure, NLC produces the controlled and predetermined drug release from NLC carriers. Likewise, NLC stays in its solid state at body temperature by controlling the measure of liquid lipids added to the dosage form. This provides an essential tool to release the drug over a prolonged time period [29].

In TDDS, molecular properties of drug plays a vital role for transdermal potential i.e., when the molecular weight of any drug is >500 Da, it is confirmed that this type of drug cannot permeate through the skin until it is changed to a nanostructured form. By comparing with other carrier molecules like creams, tinctures, emulsions and SLN, NLCs has more advantage by providing significant controlled release of drug, protection of drug that leads to good stability and very low irritation of skin [30].

1.11. Hypothesis of Research

- Simvastatin shows very low bioavailability ie. 5% ; low half life 2 hours and undergoes first pass hepatic metabolism; about 60 % of unchanged form of drug is excreted in faecal and 13 % in urine.
Carvedilol shows very low bioavailability ie. 25-35% ; and undergoes first pass hepatic metabolism; about 60 % of unchanged form of drug is excreted in faecal and 16 % in urine. Due to low solubility and the above drawbacks in pharmacokinetic datas; Simvastatin and Carvedilol has no sufficient plasma drug concentration to meet the therapeutic response. And also marketed conventional dosage form also can’t able to overcome the above drawbacks. Hence, the objective of the present research work is to develop and characterize a Nanostructured lipid carrier (NLC) enriched Transdermal Dosage form to enhance the bioavailability of Simvastatin and Carvedilol.